Philippine Herbal Pharmacopeia 2022

- Introduction
- General Notices and Requirements
- General Chapters | General Methods
- General Information
- Official Monograph
- Annexes

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Introduction

General Notices and Requirements

General Chapters | General Methods

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Annexes

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Introduction

BRIEF HISTORY AND PURPOSE OF PHILIPPINE HERBAL PHARMACOPEIA

Books on Philippine medicinal plants have been published since the 18th century, primarily by Spanish botanists and herbalists, including those by Fray Manuel Blanco. He wrote three editions of the *Flora de Filipinas* starting in 1737. In the 19th century, Dr. Trinidad H. Pardo de Tavera authored *Plantas Medicinales*, a collection of medicinal plants, their scientific and popular names, the qualities of the plant, and the ailments that can be treated with them. Another significant published work was that of Dr. Eduardo Quisumbing entitled *Medicinal Plants in the Philippines* in 1935.

The groundwork for creating an herbal formulary was established through the research on the phytochemistry of medicinal plants made by three former deans of the University of the Philippines (UP) College of Pharmacy namely: Dr. Alfredo C. Santos, Dr. Patrocinio B. Valenzuela, and Dr. Jesusa A. Concha in the 1930s and 1940s. In 1996, Dr. Conrado Dayrit, who headed the National Academy of Science and Technology (NAST), and Professor Akira Hamada, then Regional Adviser on Pharmaceuticals in the World Health Organization (WHO), agreed to pursue creating a Philippine Pharmacopeia. The Philippine Pharmacopeia I (PPI) was a joint effort of Filipino and Japanese scientists and included monographs of synthetic drugs as well as crude plant drugs. In 2004, President Gloria Macapagal-Arroyo signed E.O. No. 302, s. 2004 – Declaration and Adoption of the Philippine Pharmacopeia as the official book of standards and reference for pharmaceutical products and crude plant drugs in the Philippines. Since then, continuous research has validated the effectiveness and safety of several medicinal plants, as well as the registration of various herbal medicines developed by Philippine scientists and produced by the Philippine pharmaceutical industry. With the rise in the popularity of herbal medicines and the increase in the number of herbal products, it is imperative to develop a new pharmacopeia that defines the standards and references for these products to ensure they adhere to quality standards.

THE PHILIPPINE HERBAL PHARMACOPEIA PROJECT

Producing an herbal-only pharmacopeia has also been in the strategic plans of the Institute of Herbal Medicine-National Institutes of Health (IHM-NIH) of the University of the Philippines since September 2018. The project came to fruition when the Philippine Institute of Traditional and Alternative Health Care (PITAHC) made it its priority in November 2019. Under the leadership of PITAHC Director General Anabelle Pabiona-De Guzman, the agency fully funded the project. Cecilia C. Maramba-Lazarte, Director of IHM-NIH, led the project when it started in December 2019. The project's objectives were two-fold: 1. To create and institutionalize an official Philippine Herbal Pharmacopeia Organization consisting of the executive board, steering committee, and technical working groups; and 2. To produce and publish the first Philippine Herbal Pharmacopeia consisting of official monographs of Philippine medicinal plants and herbal medicines. The first objective is essential to ensure the continuity of the mission of standardizing quality as more and more herbal medicines are found to have therapeutic or prophylactic value. New herbal medicines are regularly being registered and added to the official monographs in the Philippine Herbal Pharmacopeia. Thus, an appropriate agency should also provide regular funding.

- The IHM and PITAHC formed the original core members and jointly created the official pharmacopeial organization, including the Executive Committee, Steering Committee (SC), and the Technical Working Groups (TWG). Experts from the PITAHC, academe, pharmaceutical industry, and other scientific disciplines are included in the committees, including Dr. Annabelle Pabiona-de Guzman, Atty. Carmencita Castro-Santos, Dr. Cecilia C. Maramba-Lazarte, Ms. Maria Lourdes Santiago, Ms. Lillibeth Chua-De Los Reyes, Dr. Nelia C. Maramba, Dr. Fabian Dayrit, Prof. Leticia Barbara B. Gutierrez, Dr. Constancio de Guzman, Dr. Francis Wade Gomez, Dr. Ellinor Roquel, Dr. Bienvenido Balotro, Dr. Alice Catabay, Dr. Marilou Nicolas, Dr. Grecebio Jonathan Alejandro, Ms. Pia Rose Belarmino, Mr. Philip Cruz,

Prof. Jocelyn Palacpac, Prof. Leslie Roces, Dr. Noel Quiming, Prof. Natividad Lacdan, Dr. Jessica Rey, and Mr. Jeff Louine Jamir Domingo.

On January 17, 2020, the then Director General of the Philippine Food and Drug Administration (FDA), Dr. Rolando Enrique Domingo, formally expressed his support for the project and assigned expert representatives from the FDA to be included in the committees: Dir. Jocelyn Balderrama, Ms. Maria Lourdes Samonte, initially Maria Lourdes Santiago, and later on Dir. Jesusa Joyce Cirunay.

On May 27, 2020, Dr. Domingo presented the Philippine Herbal Pharmacopeia project to the Department of Health (DOH) Executive Committee, which endorsed the PHP proposal. Thus, the project proceeded promptly.

There are several differences between the monographs in Philippine Pharmacopeia I compared to the Philippine Herbal Pharmacopeia, particularly in the selection of plants to be included. In PP1, the crude plant drugs included were based on their folkloric use in Philippine traditional medicine. In the PHP, the following criteria were used by the Steering Committee (SC) to prioritize which medicinal plants and herbal medicines would be included:

1. The medicinal plant must be indigenous or endemic and used by the Philippine community; OR
2. It has been traditionally used for at least 50 years in the Philippines; AND
3. With proven efficacy and safety based on FDA Administrative Order No. 172 s. 2004 Guidelines for Registration of Herbal Medicines and is either
    a. Registered as an herbal medicine with the Philippine FDA; or
    b. One of the ten medicinal plants recommended by DOH; or
    c. Approved by the steering committee based on the submitted studies.

- Unfortunately, like many projects that are in full swing at this time, the COVID-19 pandemic posed many challenges for the project, and solutions were sought. Face-to-face meetings were not allowed when alert levels were 2 or higher. Several committee members were senior citizens and were at high risk for severe disease. Thus, online meetings were held to ensure the safety of the organization members. Due to the enforcement of lockdowns for about 4 months, major farm operations were stopped, such as weeding, watering, plant propagation, and maintenance. Because workers were not allowed to enter the area, all newly propagated plants (mostly annuals) such as *oregano*, *yerba buena*, *ampalaya*, and *ulasimang bato* died of non-irrigation. Growth of standing perennials such as *lagundi*, *tsaang gubat*, *bayabas*, *ipil-ipil*, *akapulko*, and *sambong* was also affected due to the overgrowth of weeds and pest infestation. With the quarantines and lockdowns in Metro Manila and Laguna, access to the laboratories at UP in Manila, Los Baños, and Diliman have been limited. Meetings of the Technical Working Groups 1 and 2, Steering Committee, and Executive Committee were held online, via Zoom. Special permits, necessary to gain access to the laboratories of UP in Diliman, Manila, and Los Baños, had to be obtained then. The completion of the project was delayed because of these series of pandemic restrictions. It was only when the lockdown was lifted that the rehabilitation of the farm area was undertaken, paving the way for the resumption of farm operations.

This book, created by the combined efforts of experts from the academe, PITAHC, FDA, pharmaceutical industry, and other scientific disciplines, is another landmark event in promotion of the rational and scientific use of Philippine medicinal plants.

Project Working Group

The first edition of the Philippine Herbal Pharmacopeia is the result of the research project funded by the PITAHC with the NIH-IHM as project proponent.

The project's principal investigator was Dr. Cecilia Maramba-Lazarte, Director of IHM-NIH. She is part of the Executive Committee along with: Director General of PITAHC Dr. Annabelle P. De Guzman, former Director of FDA Dr. Rolando

Domingo, PITAHC Legal Officer Atty. Carmencita Castro-Santos, and the Office of the Government Corporate Counsel (OGCC) representative.

The Executive Committee is tasked to:

- Provide overall policy direction to the project
- Endorse the work plan of the Philippine Herbal Pharmacopoeia Organization to the Secretary of Health
- Establish funding resources
- Review and approve the recommendations of the TWGs
- Approve the recommendations of the Steering Committee
- Ensure the continued development and the revisions of the Philippine Herbal Pharmacopeia

The project's Steering Committee is composed of experts from the academe, government agencies, herbal drug industry, and the scientific disciplines. The initial members of the Steering Committee were (a) Maria Lourdes Santiago (FDA), (b) Ms. Lillibeth Chua- De los Reyes (PITAHC), (c) Dr. Nelia C. Maramba (academe), (d) Dr. Fabian Dayrit (academe), (e) Prof. Leticia Barbara-B. Gutierrez (academe), (f) Dr. Constancio de Guzman (academe), (g) Dr. Francis Wade Gomez (Industry), (h) Dr. Elinor Roquel (industry).

The Steering Committee is tasked to:

- Supervise and support the work of the Technical Working Groups (TWGs)
- Review issues and recommend activities to be undertaken by the TWGs including, but not limited to:
  - Defining the different tests and references to be included in the Philippine Herbal Pharmacopeia based on the WHO Annex 7 Good Pharmaceutical Practices: Chapter on Monograph in Herbal Medicine - Technical Report Series 1010, 2018
  - Prioritizing the forty-five (45) herbal plants to be included in the Philippine Herbal Pharmacopeia
- Review and report work plans and requirements of the TWGs to the Executive Board
- Monitor implementation of work plans and submission of reports of the TWGs
- Recommend the composition of the TWGs
- Coordinate activities of the TWGs

There are two Technical Working Groups in the project. The Technical Working Group 1 was in charge of preparing the documents for the General Considerations. The Technical Working Group 2 prepared the herbal drug monographs.

The Technical Working Groups are tasked to:

- Formulate the work plans and conduct activities towards the development of the Philippine Herbal Pharmacopeia
- Coordinate research activities in order to develop standards of identity, purity, and quality for herbal medicines and medicinal plants
- Provide scientific and technical support to the development and completion of the Philippine Herbal Pharmacopeia
- Submit to the Executive Board reports of accomplishments and other relevant documents on a regular basis.

The Secretariat is tasked to perform the following:

- Provide communication conduit to and from the committees and board
- Maintain and organize official documents

- Manage and organize calendar and meeting schedules
- Provide direct assistance to each Committee in the provision of outputs
- Facilitate conferences and provide transcription services during meetings
- Tracks and facilitates activities and processes.
- Manage electronic mail and phone correspondence to communicate and provide deliverables
- Maintain, update, and organize record files and documents
- Preserve and update personnel files and other related documents
- Furnish documents for processing

Project Objectives and Purpose

The general objective of the research project is to develop and produce a Philippine Herbal Pharmacopeia containing 45 monographs of medicinal plants and herbal drug products which will serve as standards and reference for the determination of the identity, purity, and quality of the herbal drugs.

Its specific objectives are:

- To advocate for the establishment of the official pharmacopeial organization which includes the Executive Committee, Steering Committee, and the Technical Working Groups.
- To generate the necessary administrative orders and legal procedures to address the requirements of the creation of the pharmacopeia
- To prioritize the medicinal plants and herbal medicines which will be included in the Philippine Herbal Pharmacopeia based on the criteria set by the Steering Committee;
- To collect and process the medicinal plants and perform the appropriate tests specified by the committees.

The first edition of the Philippine Herbal Pharmacopeia was completed in June 2022. It consists of monographs of dried plant parts, powdered plant parts, extracts, and dosage forms of thirty-eight (38) herbal drugs and the Chapters on the General Considerations. The Chapters on General Considerations provide guidance and information for the users of the pharmacopeia and consists of the Introduction, General Notices and Requirements, General Statements, General Chapters General Methods, and General Information.

AUTHORIZED STATUS

The Philippine Herbal Pharmacopeia is the first edition of herbal drug monographs funded and initiated by the PITAHC and the NIH-IHM, UP Manila as the implementing agency. It is published with the endorsement of the DOH Secretary.

LEGAL RECOGNITION

The Philippine Herbal Pharmacopeia's legal recognition as the official book of standards and reference of herbal drugs in the Philippines is derived from the recognition of the Pharmacopeia by the Philippine FDA of the DOH.

ACCEPTANCE CRITERIA

Acceptance criteria are numerical limits, ranges, or other suitable measures for acceptance of the results of analytical procedures. The acceptance criteria indicated in the Philippine Herbal Pharmacopeia monograph allow for analytical error, for unavoidable variations in manufacturing, and for deterioration to an extent considered acceptable under practical conditions. Producing an article to stricter criteria than those specified in the monograph does not constitute the basis for a claim that the article "exceeds" the compendial requirements.

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General Notices and Requirements

The *General Notices and Requirements* provide the basic rules for the interpretation and application of the standards, tests, assays, and other specifications of the Philippine Herbal Pharmacopeia.

Section 1

TITLE

The full title of this book, including its supplements, is the Philippine Herbal Pharmacopeia 2022. This title may be abbreviated to PHP2022. In case when the term PHP is used without further qualification during the period when this Pharmacopeia is officially effective, it refers to the Philippine Herbal Pharmacopeia 2022 (PHP2022).

OFFICIAL STANDARDS AND REFERENCES

The requirements stated in the monographs of the Philippine Herbal Pharmacopeia apply to articles that are intended for medicinal use but not necessarily to articles that may be sold under the same name for other purposes. An article intended for medicinal use that is described by means of an official title must comply throughout its assigned shelf life (period of validity). The subject of any other monograph must comply throughout its period of use.

The Philippine Herbal Pharmacopeia is a book of standards and reference for the determination of the identity, purity, and quality of herbal products and crude plant products. For articles not covered in PHP2022, other references may be used.

MONOGRAPH NOMENCLATURE

The official title of each herbal substance and herbal product is given in English. Any subsidiary title included in the monograph is accompanied by its chemical name, whenever applicable.

The first letter of all the important words in the Pharmacopeial title begins with capital letters.

OFFICIAL REQUIREMENTS

The official requirements for the Pharmacopeial articles are outlined in the *General Notices and Requirements*; *Individual Monographs*; *General Rules on Herbal Products*; *General Tests and Assays*, *Reagents*, *Indicators, and Solutions*; and *Reference Standards of this Pharmacopeia*. However, General Information should not be taken as indicating standards for conformity.

In the monograph of crude plant drugs, the following do not constitute official requirements but are included as added information to help the characterization of the crude plant drugs:

- Scientific Names;
- Selected Common Names;

- Morphological Description of the Plant;
- Botanic characteristics of the Underground Plant Part; and
- Traditional Uses.

Histology of the crude plant drug and botanical characteristics of the powdered drug remain to be part of the official requirements for crude plant drugs.

Section 2

MONOGRAPH REQUIREMENTS

SCIENTIFIC NAMES, COMMON NAMES & DESCRIPTION OF THE PRODUCT

The official title of crude plant drugs starts with the Latin binomial name of the plant source in the genitive case followed by the Latin name of the plant part in the nominative case. The "Official Title" of the crude plant drug is synonymous with the "Pharmacopeial Title". When used in the label of a crude plant drug, it means that the article meets the standards specified in the monograph.

If an official title contains an adjective, the noun in the nominative case precedes the adjective which must agree with the noun in case, number, and gender.

The National Title which is the most common name of the plant is printed below the Official Title of the crude plant drug.

MASS, MEASUREMENT AND UNITS OF POTENCY

The International System of Units (SI) of Mass and Measures is used in this Pharmacopeia. The SI metric and other units, and the symbols commonly employed are listed in the section on *Units of Measurements*.

► Metric or International System of Units (SI)

In pharmacy and medicine, we most often need to measure or use units of length, mass, and volume. The basic unit of length in SI is the meter, although in pharmacy we more often use the centimeter, which is 0.01 meter (= 1/100 of a meter).

The basic unit of volume in SI is the cubic meter (m^3), but the liter (L), although not an SI unit, is approved for use in SI for measuring liquid volume. The liter is the fundamental unit of volume used in pharmacy and medicine and is essentially the volume of 1,000 cubic centimeters (cm^3). The abbreviation "cc" is sometimes used for cubic centimeter, but this notation does not follow SI conventions. Moreover, its use is discouraged since a poorly written "c" can be mistaken as "U" (units).

Although the official basic unit of mass in SI is the kilogram, the gram (g) has been used as the basic stem unit. One gram is the mass of 1 cm^3 of water at 4°C. Because 1 mL (1 mL = 0.001 L) is equal to 1 cm^3, 1 mL of water also has a mass of 1 g. [Note that kilograms and grams are units of mass (not weight), even though in practice we usually use the term weight when we actually mean mass. In this Pharmacopeia, we will use the terms weight and mass interchangeably, although technically weight is a measure of force and is mass times gravitational acceleration.]

Other metric units of length, volume, and weight are achieved by applying prefixes to an appropriate stem unit: meter (m), liter (L), or gram (g). The prefix specifies a power of ten that is multiplied by the stem unit. For example, the prefix milli (m) is associated with a 10^{-3} multiple, so that a millimeter (mm) is 10^{-3} meters, a milligram (mg) is 10^{-3} grams, and a milliliter (mL) is 10^{-3} liters. Prefixes for SI units, together with their associated powers of ten and their symbols, are shown in ***Table 2.1***

Table 2.1. SI Prefixes, Values and Symbols

POWER OF TEN	PREFIX	SYMBOL
10^{12}	tera	T
10^{9}	giga	G
10^{6}	mega	M
10^{3}	kilo	k
10^{2}	hecto	h
10	deca	da
1	no prefix (basic unit)	-
10^{-1}	deci	d
10^{-2}	centi	c
10^{-3}	milli	m
10^{-6}	micro	µ
10^{-9}	nano	n
10^{-12}	pico	p

There are a number of basic rules to observe in the use of the SI units and symbols. Some important examples are listed here.

a. The short forms for the dimension units, such as "cm" for centimeter, are called symbols rather than abbreviations.

b. The letter case, either uppercase (capitalized) or lowercase, is important. The lowercase "m" for milli, for example, signifies 10^{-3}, whereas the uppercase "M" for mega designates 10^{6}. Uppercase letters are used for symbols indicating powers of 10^{6} and higher.

c. Symbols are always written in singular form but, when the word for a unit is used, the plural form is used when appropriate. For example, write "5 mL" (not "5 mLs"), but write "5 milliliters."

d. There should always be a space between the numeric digits of a quantity and its SI symbol (e.g., "5 g" rather than "5g").

e. The symbols are never followed by periods unless a symbol is at the end of a sentence.

f. While the SI symbol for micro is the lowercase Greek letter mu (µ), this symbol should not be used when expressing doses (e.g., use mcg instead of µg), because µg can easily be misread as mg.

g. When a symbol is a compound unit, and it is a quotient of two units, that quotient may be designated with a solidus (forward slash mark, "/") or a negative exponent (e.g., g/cm^3 or g cm^{-3}). The letter "p" is not to be used for the word per, but per may be written out (e.g., 5 milligrams per milliliter). (Note: In pharmacy and medicine, when writing dose concentrations, the use of the solidus is not recommended because of medication errors that have resulted from reading it as a number one. In this text, the word per is used in place of the solidus in these circumstances.)

Table 2.2. SI-Metric Units and Symbols

SI-METRIC UNIT	SYMBOL
meter	m
centimeter	cm
millimeter	mm
liter	L or l
milliliter	mL or ml
kilogram	kg
gram	g
milligram	mg
microgram	µg (mcg for drug orders)
hour	hr
second	s
cubic centimeter	cm^3 (mL for drug orders)
degree Celcius	^{0}C
kelvin	K

Table 2.3. SI-Metric Measures Commonly Used In Pharmacy and Medicine

SI-METRIC UNIT	EQUIVALENT SI-METRIC UNIT
METRIC MEASURES OF LENGTH	
100 centimeters (cm)	1 meter (m)
10 millimeters (mm)	1 centimeter (cm)
1,000 micrometers (µm)	1 millimeter (mm)
METRIC MEASURES OF VOLUME	
1,000 milliliters (mL)	1 liter (L or l)
1,000 microliters (µL)	1 milliliter (mL)
METRIC MEASURES OF WEIGHT	
1,000 grams (g)	1 kilogram (kg)
1,000 milligrams (mg)	1 gram (g)
1,000 micrograms (ug or mcg)	1 milligram (mg)

FORMULATION AND DOSAGE

Herbal formulation is a dosage form consisting of one or more herbs or processed herb(s) in specified quantities to provide specific medicinal, nutritional, cosmetic benefits, and/or other benefits meant for use to diagnose, treat, mitigate diseases of human beings or animals and/or to alter the structure or physiology of human beings or animals. Herbal formulations are obtained by subjecting herbal substances to processes such as extraction, distillation, expression, fractionation, purification, concentration or fermentation. These include comminuted or powdered herbal substances, tinctures, extracts, essential oils, expressed juices and processed exudates.

Dosage forms commonly employed for pharmaceuticals, foods or cosmetics may be used to formulate one or more herbs or processed herbs. Dosage forms known in traditional medicines may also be adopted for preparing herbal formulations, either for external use or for internal administration. Adequate consideration for uniform distribution of herbs or processed herbs as well as their stability in dosage form will be provided during formulation development. Herbal formulation should be labeled to comply with relevant labeling requirements of the Philippine FDA. Additionally, adequate information will be provided on the label of the formulations such as the name of the herb, parts used, nature and type of extract or processed herb used, extraction ratios, quantity per unit dose or per serving, name(s) of inert excipients used and any preservatives added.

Inclusion of the monograph of an herbal formulation in the Pharmacopeia does not mean it is legally approved as a drug. Monograph of an herbal formulation in the Pharmacopeia provides qualitative and quantitative standards of quality for the formulation of its use as an herbal drug, food supplement, and as a cosmetic. Each use needs to comply with applicable regulations.

Herbal dosage forms are the physical form (liquid, solid, semi-solid) of herbal products produced from herbs, with or without excipients, in a particular formulation (such as capsules, tablets, and ointments). They are produced either from herbal materials (such as dried leaves or juices) or herbal preparations (such as extracts). The type of formulation given may depend upon the patient's age, sex, and health condition and is specific to particular routes of administration. The processes for the preparation of these dosage forms are beyond the scope of the Pharmacopeia.

LABORATORY TEMPERATURE

Unless otherwise stated, all temperatures in this Pharmacopeia are expressed in degrees Celsius (°C) and all measurements made are in 25°C.

Whenever an analytical procedure indicates temperature without a numerical figure, the general terms used have the following meaning:

Deep freeze	below -65°C
In a freezer	below -10°C
Cold	2°C to 8°C
Cool	>8°C to 15°C
Room temperature	20°C to 30°C

Analytical procedures are carried out at room temperature, unless otherwise stated.

CONCENTRATION

Molarity uses the symbol M preceded by a number which represents the number of the moles of the designated solute contained in an amount of the designated solvent that is sufficient to prepare 1 liter of solution. Normality uses the symbol N preceded by a number, which represents the number of equivalents of the designated solute contained in an amount of the designated solvent

that is sufficient to prepare 1 liter of solution.

PERCENTAGE MEASUREMENTS

Percentage (%) concentrations are expressed as follows:

- **Percent mass in mass (m/m)**

  Expresses the number of grams of a constituent in 100 g of solution or mixture.

- **Percent mass in volume (m/v)**

  Expresses the number of grams of a constituent in 100 mL of solution and is used regardless of whether water or another liquid is the solvent.

- **Percent volume in volume (v/v)**

  Expresses the number of mL of a constituent in 100 mL of solution.

SOLUTION

If the name of the solvent is not stated, the term "solution" indicates solution in water.

Unless otherwise specified in the individual monograph, all solutions called for in tests and assays are prepared with the United States Pharmacopeia (USP) purified water/distilled or demineralized water of adequate purity.

An expression such as "(1/10)" means that 1 part by volume of a liquid is diluted with, or 1 part by mass of a solid is dissolved in sufficient quantity of the diluent or solvent to make the volume of the finished solution 10 parts by volume.

An expression such as "(20:5:2)" means that the respective numbers of parts, by volume, of the designated liquids are to be mixed.

The notation "VS" after a specified volumetric solution indicates that such solution is standardized in accordance with the directions given in the individual monograph or under volumetric solutions in the section *Reagents, Indicators and Solutions* as differentiated from solutions of approximate normality or molality.

Is a standardized solution of a specific concentration is called for in a test or an assay, a solution of other normality or molarity may be used, provided that (a) an allowance is made for the difference in concentration and that (b) the error of measurement is not increased thereby.

APPARATUS

A specification for a definite size or type of container or apparatus in a test or assay is given solely as a recommendation. If the volumetric flask or another exact measuring, weighing, or sorting device are specified, these or other equipment of at least equivalent accuracy will be employed. If a low-actinic or light-resistant containers are specified, clear containers that have been rendered opaque by application of a suitable coating or wrapping may be used.

Graduated glass apparatus used in analytical operations should comply with Class A requirements of the appropriate international standard issued by the International Organization for Standardization (ISO).

CENTRIFUGE

If the use of a centrifuge is indicated, unless otherwise stated, the directions are predicated upon the use of apparatus having an effective radius of about 20 cm and driven at a speed sufficient to clarify the supernatant layer within 15 mins.

THERMOMETERS

Standardization of thermometers is performed according to established testing frequency with a temperature standard traceable to the US National Institute of Standards. Refer to the current issue of the American Society for Testing and Materials Standard E1.

GLASSWARE

Use glassware that has been scrupulously cleaned by a method that will remove organic matter. Use purified water for the final rinse of the glassware. The use of volumetric glassware that does not comply with Class A requirements of the appropriate international standards of the ISO is acceptable if the validation or the system suitability test of the method using such glassware has experimentally demonstrated that the method is suitable for the intended purpose.

WEIGHTS AND BALANCES

Unless otherwise stated, when a substance is to be "accurately weighed" for assay, the weighing should be performed with a weighing device whose measurement of uncertainty (random plus systematic error) does not exceed 0.01%. Measurement of uncertainty is satisfactory if three times the standard deviation of not less than 10 replicate weighings divided by the amount weighed does not exceed 0.001.

Unless otherwise stated, the weighing for the titrimetric limit tests should be performed to provide the number of significant figures in the mass of the analyte that corresponds to the number of significant figures on the concentration of the titrant.

PHYSICAL MEASUREMENT

Where an instrument for physical measurement, such as a spectrophotometer, is specified in a test or assay by its distinctive name, another instrument of equivalent or greater sensitivity and accuracy may be used. In order to obtain solutions having concentrations that are adaptable to the working range of the instrument being used, solutions or proportionately higher or lower concentrations may be prepared according to the solvents and proportions that are specified for the procedure.

PROCEDURES

Laboratories conducting assays or procedures described in the Pharmacopeia should follow Good Laboratory Practices (GLP). When examined according to the test procedures described in the compendium, every compendial article in commerce should comply with all the requirements described in the said monograph.

FOREIGN SUBSTANCES AND IMPURITIES

Tests for the presence of foreign substances and impurities are provided to limit such substances to the amount that is acceptable under conditions in which the article is customarily employed.

IDENTIFICATION TESTS

Identification tests are provided for the verification of the identity of the substance described in the monograph. Such tests, however specific, are not necessarily sufficient to establish proof of identity but failure of an article taken from a labeled container to meet the requirements of a prescribed identification test indicates that the article may be mislabeled and will require confirmatory test(s).

TESTS AND ASSAYS

The tests and assays described are official methods that the standards of the Pharmacopeia depend on. The analysts are not precluded from employing alternative methods, including methods of microanalysis, in any tests and assays if they have been proven to give results of equivalent accuracy. Local reference materials may be used for routine analysis, provided these are calibrated against the official reference materials. In the event of doubt or dispute, the methods of analysis, the reference materials and the reference spectra of the Pharmacopeia are the only authority.

BLANK DETERMINATION

If the blank determination is to be conducted, use the same quantities of the same reagents treated in the same manner as the solution or mixture containing the portion of the substance under assay or test, but with the substance omitted.

CALCULATION OF RESULTS

The results of the assay should be calculated to one decimal more than indicated in the requirement and then rounded up or down as follows:

1. If the last figure calculated is 5 to 9, the preceding figure is increased by 1;
2. If it is 4 or less, the preceding figure is left unchanged.

Other calculations, for example in the standardization of volumetric solutions, should be carried out in a similar manner.

In the determination of ashes, extractable material, bitterness, hemolytic activities, tannoids and swelling index and in chemical assays, calculations must be made with reference to the undried sample.

If the material has to be dried before it can be reduced to a powder for use in a determination, a correction must be made to take into account the loss on drying, and the amount of active principle calculated with reference to the undried sample.

For herbal drugs, the sulfated ash, total ash, water-soluble matter, alcohol-soluble matter, water content, content of essential oil and content of active principle are calculated with reference to the drug that has not been especially dried, unless otherwise specified.

REAGENTS

Reagents used should conform to the specifications set in the current edition of Reagent Chemicals published by the American Chemical Society (ACS). The reagents required for the tests and assays of the Pharmacopeia are defined by their nature, degree of purity and the strengths of the solutions to be made from them. The requirements set are not intended to imply that the materials are suitable for use in medicine; reagents not

covered by monographs in the pharmacopeia should not be claimed to be of quality. The term "analytical reagent grade of commerce" implies that the chemical is of a high degree of purity wherein the limits of various impurities are known. If the direction is to use a "general laboratory reagent grade of commerce," use of a chemically pure grade material, is not necessarily required to be tested for limiting or absence of certain impurities.

There are many different chemical grading standards. Most chemicals are labeled with a grade which generally indicates the purity of the chemical. https://labproinc.com/pages/common-chemical-grades

ACS is a chemical grade of the highest purity and meets or exceeds purity standards set by the American Chemical Society (ACS).

Reagent High is generally equal to ACS grade and is suitable for use in many laboratory and analytical applications.

USP is a chemical grade of sufficient purity to meet or exceed requirements of the U.S. Pharmacopeia (USP); acceptable for food, drug, or medicinal use; may be used for most laboratory purposes.

NF is a grade of sufficient purity to meet or exceed the requirements of the National Formulary (NF).

HPLC grade is for High-performance Liquid Chromatography and is used to separate, recognize, and calculate each chemical compound in a mixture.

LP (Low Particulate) grade refers to a low level of particulate matter found in a chemical.

REFERENCE STANDARDS

Reference standards are authentic specimens that have been verified for suitability as comparison standards in Pharmacopeial tests and assays.

Whenever Reference Standards is referred to in an assay or test in the Pharmacopeia, abbreviate the words "Reference Standards" to "RS."

If a test or an assay calls for the use of a Pharmacopeial article as a material standard of reference rather than a Reference Standard, use a substance meeting all of the requirements of the monograph for that article.

CONTAINERS

The container is the device that holds the article either in the form of the raw material or of the drug product. The closure of the container, including the stopper, the cap, the attached dropper, etc., is considered a part of the container.

The immediate or primary container is the one that is in direct contact with the article at all times.

The container should be clean. No extraneous matter should be introduced into it or into the article placed in it. It must not interact physically or chemically with the article which it holds so as not to alter the article's quality, purity, or therapeutic potency. Environmentally-friendly containers are preferred and should be used whenever available.

► Well-closed Container

A well-closed container protects the contents from extraneous solids and from loss of the article under ordinary or customary conditions of handling, shipment, storage, and distribution.

► Tight Container

A tight container protects the contents from contamination by extraneous liquids, solids, or vapors, from loss of the article, and from efflorescence, deliquescence, or evaporation under ordinary or customary conditions of handling, shipment, storage, and distribution, and is capable of tight re-closure. If a tight container is specified, it may be replaced by a hermetic container for a single-dose of an article.

► Hermetic Container

A hermetic container is impervious to air or any other gas under ordinary or customary conditions of handling, shipment, storage, and distribution.

► Light-resistant Container

A light-resistant container protects the contents from the effects of light through the specific properties of its material, including any coating applied to it.

If the directions in the individual monograph is "to protect from light", use light-resistant container for preservation.

► Tamper-evident Container

A tamper-evident container is so sealed that the contents cannot be used without destroying the seal.

LABELING

The term "labeling" refers to all labels and other written, printed, or graphic matter put on an immediate container of an article or any package or wrapper in which the article is enclosed. It does not include outer shipping container.

The label on the container states the Official Title, which is made up of the Latin binomial name of the plant source followed by the part of the plant which constitutes the crude plant drug. Below it is the National Title, which is made up of the best-known Philippine name (Filipino name) of the plant source, followed by the part of the plant which constitutes the crude plant drug.

Every article in the Pharmacopeia should bear one or more labels indicating the identity and certain pertinent characteristics. The label(s) should be affixed or firmly fastened to, or indelibly written on, the article's immediate container and its packaging, if any. The label should comply with the current labeling requirements promulgated by the DOH.

A shipping container or a shipping package is exempted from the labeling requirements of this Pharmacopeia.

Labeling requirements may not apply when the article is supplied in compliance with the compounding and dispensing of a medical prescription.

STORAGE TEMPERATURES FOR DRUG SUBSTANCES AND DRUG PRODUCTS

- **Temperature**

  Materials that need to be stored at temperatures other than room temperature should be labeled accordingly.

- **Humidity**

  Low humidity may be maintained, if necessary, by the use of a desiccant in the container, provided that direct contact with the product is avoided. Care must be taken when the container is opened in damp or humid conditions.

- **Protection from light**

  Medicinal plant materials requiring protection from light should be kept in a light-resistant container that—either by reason of the inherent properties of the material from which it is made or because a special coating has been applied to it—shields the contents from the effects of light. Alternatively, the container may be placed inside a suitable light-resistant (opaque) covering and/or stored in a dark place.

The conditions of storage are defined by the following terms:

- **Freezing**

  Any temperature maintained thermostatically below -10°C.

- **Cold**

  Any temperature between 2°C and 8°C.

- **Cool**

  Any temperature between 8°C and 15°C. An article for which storage in a cool place is directed may, alternatively, be stored in a refrigerator, unless otherwise specified by the individual monograph.

- **Room Temperature**

  Any temperature between 20°C and 30°C.

- **Controlled Room Temperature**

  A temperature maintained thermostatically that encompasses the usual and customary working environment of 20°C to 25°C that results in a mean kinetic temperature calculated to be not more than 25°C. It allows for excursions between 15°C and 30°C that are experienced in pharmacies, hospitals, and warehouses. Articles may be labeled for storage at "Controlled room temperature" or at "up to 25°C."

  An article directed to be stored at "controlled room temperature" may alternatively be stored in a "cool place," unless otherwise stated in the individual monograph or on the label.

- **Warm**

  Any temperature between 30°C and 40°C.

- **Excessive heat**

  Any temperature above 40°C.

- **Protection from Freezing**

  If there is risk of an article's loss of strength or potency, or the destructive alteration of its characteristics, or the risk of breakage of its container, the container must bear appropriate instructions to protect the article from freezing.

► **Storage under non-specific conditions**

For articles—regardless of quantity—with no specific directions or limitations in the individual monograph, conditions for storage and distribution include protection from moisture, freezing, and excessive heat.

FRESHLY AND RECENTLY PREPARED

The direction "preparation must be freshly prepared" means that the article must be made not more than 24 hours before it is issued for use. The direction "preparation should be recently prepared" means that deterioration is likely if the preparation is stored for longer than about four weeks at 15°C - 25°C.

MACROANALYSIS

► **Size of Cut**

Medicinal plant materials are used either whole, or in cut or powdered form.

Cut medicinal plant materials are prepared by cutting or crushing the plant into small pieces. The cut is graded according to the aperture size of the mesh of the sieve through which the material will pass. They are listed in ***Table 2.4.***

Table 2.4. Aperture Size

Aperture size (mm)	
Coarse cut	4.00
Medium cut	2.80
Fine cut	2.00

► **Physical measurements**

Organoleptic and macroscopic descriptions of the crude plant or herbal medicine include the appearance, texture, color, odor and taste of the subject.

Macroscopic study must include the morphological structure of each plant part as described in individual monographs.

UNITS OF MEASUREMENTS

The International System of Units (SI) of Mass and Measures is used in this Pharmacopeia. The SI metric and other units, and the symbols commonly used are listed in ***Table 2.5***.

Table 2.5. Units of Measurements

QUANTITY	NAME	SYMBOL	VALUE IN SI UNIT
Time	minute	min	60 s
	hour	hr	3,600 s
Volume	microliter	µL	1/1,000,000 of a liter
	milliliter	mL	1/1,000 of a liter
	liter	L	1,000 mL
Mass	picogram	pg	1/1,000,000,000,000 of a gram
	microgram	µg	1/1,000,000 of a gram
	milligram	mg	1/1,000 of a gram
	gram	g	1,000 mg
	kilogram	kg	1,000 g
	metric ton	t	1,000 kg
Length or width	nanometer	nm	1/1,000,000,000 of a meter
	micrometer	µm	1/1,000,000 of a meter
	millimeter	mm	1/1,000 of a meter
	meter	m	1,000 mm
Temperature	centigrade	C	272 deg K
Electricity	hertz	Hz	
	kilohertz	kHz	1000 Hz
	megahertz	MHz	1,000,000 Hz
	volt	V	
	millivolt	mV	1/1,000 volts
Pressure	pounds per square inch	psi	
	Pascal	Pa	
	kilopascal	kPa	1,000 pascal
Concentration	Mole	M	moles per liter
	millimole	mmole	
	gram equivalent	Eq	
	milliequivalent	mEq	

Section 3

TERMINOLOGIES

ABOUT

In stating the appropriate quantities to be taken for assays and tests, the use of the word "about" means a quantity within 10% of the specified mass or volume. However, the mass or volume taken is accurately determined and the calculated result is based on the exact amount taken. The same tolerance applies to specific dimensions.

ACCURATELY MEASURED AND ACCURATELY WEIGHED

Expressions such as "25.0 mL", and "25.0 mg" used with respect to volumetric or gravimetric measurements mean that the quantity is to be "accurately measured" and "accurately weighed" within the limits stated under the *Volumetric Apparatus* and *Weights and Balances*.

ALCOHOL

If "alcohol" or dehydrated alcohol" is called for in formulas, USP alcohol or USP dehydrated alcohol may be used.

If "alcohol" is called for in tests and assays, ACS reagent grade "ethyl alcohol" or USP "alcohol" should be used.

If "absolute or anhydrous Alcohol" is called for in tests and assays, ACS reagent grade "absolute ethyl alcohol" or USP "dehydrated alcohol" should be used.

CONCOMITANTLY

The term "concomitantly" used in such expressions as "concomitantly determined" or "concomitantly measured" in directions for assays and tests, means that the determinations or measurements should be performed simultaneously or at the same time.

DESICCATOR

The term "in a desiccator" specifies the use of an airtight container of suitable size and design that maintains an atmosphere of low moisture content through the use of silica gel or other suitable desiccant.

A "vacuum desiccator" is one that maintains the low-moisture atmosphere at a reduced pressure of not more than 2.67 kPa or at the pressure designated in the individual monograph.

DILUTION

Dilution is a method by which a solution is diluted "quantitatively and stepwise." This means an accurately measured portion is diluted by adding water or other solvent in the proportion indicated, in one or more steps.

The choice of apparatus used should take into account the relatively larger errors generally associated with using small-volume volumetric apparatus.

DRYING TO CONSTANT MASS

The term "dried to constant mass" means that the drying process should continue until the results of the two consecutive weighings do not differ by more than 0.50 mg per g of substance taken, provided that the second weighing was done after an additional hour of drying at the prescribed conditions.

In "drying to constant mass," the weight of the material to be dried should be specified, preferably by % change in weight. The significance of a weight change of 0.50 mg depends on the initial weight.

FILTRATION

If the term "filter" is used without further qualification, this means that the liquid should be filtered through suitable filter paper or equivalent device until the filtrate is clear.

IDENTIFICATION

This refers to the test to identify the active ingredient(s) of the drug based on its specific property.

IGNITION TO CONSTANT MASS

The term "ignite to constant mass" means that the ignition should continue until the results of the two

consecutive weighings do not differ by more than 0.50 mg per g of substance taken, provided that the second weighing was done after an additional 15 min of ignition period.

In "ignition to constant mass," the weight of the material to be dried should be specified, preferably by % change in weight. The significance of a weight change of 0.50 mg depends on the initial weight.

INDICATORS

If the use of a Test Solution (TS) as an indicator is specified in a test or an assay, approximately 0.2 mL, or 3 drops of the solution should be added, unless otherwise directed.

LOGARITHMS

Logarithms used in the Pharmacopeia are to be base 10 and are denoted by the abbreviation "log."

MICROBIAL STRAINS

If a microbial strain is cited and identified by its American Type Culture Collection (ATCC) catalog number, the specified strain should be used directly or, if subcultured, should be used for not more than five passages removed from the original strain.

NEGLIGIBLE CHANGE IN WEIGHT

The term refers to a quantity not exceeding 0.50 mg. The weight of the metrified should be specified and preferably, the change in weight expressed by "% change in weight." The significance of a weight change of 0.50 mg depends on the initial weight.

ODOR

Terms such as "odorless," "practically odorless," "a faint characteristic odor," or any variations thereof refer to the examination—for 15 min after air exposure—of either (1) a freshly opened package of the article (for packages containing not more than 25 g) or (for larger package) (2) of a portion of about 25 g of the article that has been removed from its package to an open evaporating dish of about 100 mL capacity. An odor designation is descriptive only and is not to be regarded as a standard of purity for a particular lot of an article.

PARTS PER MILLION (PPM)

"ppm" is a dimensionless number which, strictly speaking, is: mg/kg. However, if the solution is aqueous, this is changed to: mg/L (aqueous solution).

PERCENT

If the term "percent (%) is used without qualification, this means (1) percent mass in mass for mixtures of solids and semisolids; (2) percent mass in volumes for solutions or suspensions of solids in liquids; and (3) percent volume in volume for solutions of liquids in liquids.

For example, a 1 percent solution is prepared by dissolving 1 g of a solid or semisolid, or 1 mL of a liquid, in sufficient solvent to make 100 mL of the solution.

PIPET

If the use of a pipet is needed to measure a specimen or an aliquot, the said pipet should conform to the standards set forth under *Volumetric Apparatus* or its equivalent. It should be used in such a manner that the error does not exceed the limit stated for a burette, conforming to the standards set under *Volumetric Apparatus*.

Where a "to contain" pipette is specified, a suitable, volumetric flask may be substituted.

POWDER FINENESS

The fineness (***Table 2.6***) in this Pharmacopeia expressed in descriptive terms is provided in the following table. For practical reasons, sieves are the preferred means of measuring powder fineness for most pharmaceutical purposes. Sieving is most suitable where a majority of the particles are larger than about 75 µm.

Although sieving can also be used for some powder having smaller particle sizes, the method should be validated

Table 2.6. Classification of Powder Fineness

CLASSIFICATION OF POWDER	D_{50} *SIEVE OPENING (µM)
Very coarse	>1000
Coarse	355-1000
Moderately fine	180-355
Fine	125-180
Very Fine	90-125

* The USP-NF classification of a sieve opening "d_{50}" is the sieve opening through which 50% or more of the material can pass.

SOLUBILITY

The terms used to show the solubility of Pharmacopeial substances are listed in ***Table 2.7***.

Table 2.7. Solubility

DESCRIPTIVE TERMS	ONE PART OF SOLUTE PER X PART/S OF SOLVENT
Very soluble	Less than 1
Freely soluble	From 1 to 10
Soluble	From 10 to 30
Sparingly soluble	From 30 to 100
Slightly soluble	From 100 to 1000
Very slightly soluble	From 1000 to 10,000
Practically insoluble or insoluble	Greater than 10,000

The term "dissolve" or "miscible" means that it dissolves in, or mixes in arbitrary proportion with the solvent to form a clear solution or mixture. Insoluble materials other than the drug, including fibers, should not be detected or should be practically invisible, if any.

"To dilute" means to decrease the concentration of a solution by adding an appropriate solvent. Also, the amount should be specified, such as "to dilute by half" or "to dilute to a 10% solution". A suitable graduated cylinder or measuring pipet should be used.

SPECIFIC GRAVITY

Unless otherwise stated, the basis for specific gravity basis is 25°C/25°C, i.e., the ratio of the mass of a substance in the air at 25°C to the mass of an equal volume of water at the same temperature.

TIME LIMIT

The term "time limit" refers to the time used to conduct tests and assays. 5 minutes should be used for the reaction to take place, unless otherwise specified.

VACUUM

The term "vacuum" means exposure to a pressure not exceeding 2.67 kPa, unless otherwise indicated.

WATER

The water to be used in the tests of drugs should be USP "purified water". Water used as an ingredient of official preparations meets the requirements for purified water, water for injection, or other sterile forms of water covered by monographs.

WATER AND LOSS ON DRYING

If the water hydration or adsorbed water of a Pharmacopeia article is determined by the titrimetric method, the test is generally given under the heading Water (Water Determination).

If determination is through drying under specified conditions, the test is generally given under the heading "loss on drying."

However, "loss on drying" is most often given as the heading where the loss in mass is known to represent residual volatile constituents including organic solvents as well as water.

WATER BATH

Unless otherwise specified, the term "heat on a water bath" or "heat in a water bath" means boiling in a water bath or in a steam bath at about 100°C.

Section 4

ABBREVIATIONS AND ACRONYMS

AAMI	Association for the Advancement of Medical Instrumentation
ANSI	American National Standards Institute
AOAC International	Association of Official Analytical Chemists
ACS	American Chemical Society
ASTM International	American Society for Testing and Materials
ATCC	American Type Culture Collection
BP	British Pharmacopeia
CAS	Chemical Abstracts Service
cGMP	Current Good Manufacturing Practices
CRS	BP/EP Chemical Reference Substance
DOH	Department of Health
DOH-HRT	Department of Health- Health Regulation Team
E.P.A.	U.S. Environmental Protection Agency
FCC	Food Chemicals Codex
FDA	Food and Drug Administration Philippines
ICH	International Council for Harmonisation
ISO	International Organization for Standardization
IUPAC	International Union of Pure and Applied Chemistry
JP	Japanese Pharmacopeia
NIH-IHM	National Institutes of Health-Institute of Herbal Medicine
NIST	National Institute of Standards and Technology
OGCC	Office of the Government Corporate Counsel
PCR	Polymerase Chain Reaction
PDG	Pharmacopeial Discussion Group
Ph. Eur.	European Pharmacopeia
Ph. Int.	International Pharmacopeia
PHP	Philippine Herbal Pharmacopeia
PITAHC	Philippine Institute of Traditional and Alternative Health Care
PNS	Philippine National Standard
PP1	Philippine Pharmacopeia 1st Edition
US CFR	United States Code of Federal Regulations
USP-NF	United States Pharmacopeia-National Formulary
WHO	World Health Organization

ABBREVIATIONS TEST METHODS

AAS	Atomic absorption spectroscopy
CS	Colorimetric Solutions
CTAB	Cetyltrimethylammonium bromide
cGMP	Current Good Manufacturing Practices
DNA	Deoxyribonucleic acid
ELISA	Enzyme-linked immunosorbent assay
EU	Endotoxin units
FTIR	Fourier Transform Infrared spectrometry
GC	Gas chromatography
GC-FTIR	Gas Chromatography Fourier Transform Infrared spectrometry
GC-MS	Gas chromatography-mass spectroscopy
HDPE	High-density Polyethylene
HPLC	High-pressure/performance liquid chromatography
HPTLC	High-performance thin-layer chromatography
ICP-MS	Inductively coupled plasma-mass spectroscopy
IR	Infrared
ITD	Ion Trap Detector
IU	International units
LC	Liquid chromatography
LC-MS	Liquid chromatography-mass spectroscopy
LC-NMR	Liquid chromatography-nuclear magnetic resonance
LDPE	Low-density polyethylene
LOD	Loss on drying
PCR	Polymerase Chain Reaction
PLC	Planar electrochromatography
PP RS	Philippine Pharmacopeia Reference Standards
PVC	Polyvinyl chloride
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
RH	Relative Humidity
SFC	Supercritical fluid chromatography thermal analysis
SNP	Single Nucleotide Polymorphism
SSR	Simple Sequence Repeat
TLC	Thin-layer Chromatography
TS	Test Solutions
UV/Vis	Ultra violet/Visible
VS	Volumetric Solutions

Quality Statement

The monographs developed in this book of standards have been tested and validated within the foundation of a sound quality system.

General Methods

The methods herein included are in accordance with those reported in the literature and are considered as official tests, procedures, and acceptance criteria. Each herbal preparation and drugs should include the manufacturer's specifications. The products and their raw materials should be prepared according to recognized principles of good field and manufacturing practices to ensure that products meet the requirements of each monograph and that the preparations should comply with standards until their expiration date provided they are stored as directed. The herbal preparations and drugs should also be tested and complied with the details specified in the individual monographs. The procedures used are standard methods that are validated using repeats, replicates, and statistical rejection of outliers or non-conformers, or extrapolation of results to large populations with frequency of testing and found to be statistically acceptable.

Guide to General Chapters

General chapters include *General Tests and Assays, Reagents, Indicators and Solutions, and Reference Standards*. Each section on the *General Tests and Assay* is assigned a number that appears in brackets adjacent to the section name e.g. *Foreign matter* <2>

General chapters become mandatory when referred to in a monograph, unless such reference is made to cite in a way that indicates that it is not the intention to make the text referred to mandatory but rather to cite it for information. Its use is encouraged for quick access to general tests.

General Descriptions of Herbs and Herbal Products and Preparations

A. **Herbal medicines** include herbs, herbal materials, herbal preparations and finished herbal products. If the herbal medicine is a finished, labeled medicinal product, it may contain, as active ingredient(s), aerial or underground part(s) of any or other plant material, or combination thereof, whether in crude state or as plant preparations. The herbal medicine may contain excipients in addition to the active ingredient(s).

B. **Herbs** include crude materials which could be derived from lichen, algae, fungi or higher plants, such as leaves, flowers, fruit, fruiting bodies, seeds, stems, wood, bark, roots, rhizomes or other parts, which may be entire, fragmented or powdered.

C. **Herbal materials** include, in addition to herbs, fresh juices, gums, fixed oils, essential oils, resins and dry powders or herbs. In some countries, these materials may be processed by various local procedures, such as steaming, roasting or stir baking with honey, alcoholic beverages or other materials.

D. **Medicinal** is a plant (wild or cultivated) used for medicinal purposes

E. **Active ingredients** - The herbal material(s) or the herbal preparation(s) are considered to be active ingredient(s) of herbal medicine(s). However, if constituents with known therapeutic activities are known, the active ingredients

should be standardized to contain a defined amount of this/these constituent(s).

F. **Constituents with known therapeutic activity** are substances or groups of substances which are chemically defined and known to contribute to the therapeutic activity of a herbal material or of a preparation.

G. **Markers** are chemically defined constituents of an herbal material utilized for control purposes. They may or may not contribute to the clinical efficacy. When they contribute to the clinical efficacy, however, evidence that they are solely responsible for the clinical efficacy may or may not be available. Markers are generally employed when constituents of known therapeutic activity are not known or are not clearly identified and may be used to identify the herbal material or preparation or calculate their quantity in the finished product.

There are two categories of markers:

1. Active markers are constituents or groups of constituents that are generally accepted to contribute to the therapeutic activity.

2. Analytical markers are constituents or groups of constituents that serve for analytical purposes.

H. **Traditional use of herbal medicines** refers to the long historical use of these medicines. Their use is well-established and widely acknowledged to be safe and effective and may be accepted by national authorities.

I. **Traditional medicine** is the sum total of the knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures. Whether explicable or not, it is used in the maintenance of health and in the prevention, diagnosis, improvement or treatment of physical and mental illness. The terms "complementary medicine," "alternative medicine" and "non-conventional medicine" are used interchangeably with "traditional medicine" in some countries.

HERBAL PREPARATIONS

Herbal preparations are the basis for finished herbal products and may include comminuted or cut herbal materials, or extracts, tinctures and fatty oils of herbal materials. They are produced by extraction, fractionation, purification, concentration, or other physical or biological processes. They also include preparations made by steeping or heating herbal materials in alcoholic beverages and/or honey, or in other materials.

A. **Extracts** are preparations produced by concentrating the extractive of herbal drugs. There are different kinds of extracts:

i. Viscous extracts - are liquid or semi-solid (soft extracts) herbal drug preparations. Liquid extracts contain in general 1 part by mass or volume equivalent to 1 part by mass of the dried herbal drug. These preparations are adjusted, if necessary, so that they satisfy the requirements for with content of solvent, and, where applicable, for constituents. Semi-solid extracts are obtained by evaporation or partial evaporation of the solvent used for extraction.

ii. Dry extracts - a solid preparation obtained by the evaporation of the solvent from the liquid/fluid extract. Dry extract can also be prepared by spray drying with or without the use of an adsorbent, i.e., methyl cellulose, or by drying and milling to produce a powder.

iii. Soft extracts - semi-solid material obtained by total or partial evaporation of the solvent from a liquid extract.

iv. Liquid extracts - alcoholic liquid extract produced by percolation of herbal material so that 1 mL of the fluid extract contains the extractive obtained from 1 g of the herbal material.

Unless specified, extracts are prepared by pulverizing herbal drugs to a suitable size. These are extracted for a certain period of time with suitable solvents by means of cold extraction or warm extraction, or by percolation. The extractive is filtered, and the filtrate is concentrated or dried by a suitable method to make a millet with jelly-like consistency for the viscous extracts, or to make crushable solid masses, granules or powder for the dry extracts.

B. **Pills** are spherical preparations intended for oral administration. These are prepared by mixing drug substance(s) uniformly with diluents, binders, disintegrators or other suitable excipient(s) and rolling them into spherical form using a suitable method. They may be coated with a coating agent using an appropriate method.

C. **Spirits** are fluid preparations produced by dissolving volatile drug substance(s) in ethanol or a mixture of ethanol and purified water.

D. **Infusion and Decoction** are fluid preparations obtained by macerating herbal drugs in water.

They are prepared by cutting the drug (s) into the sizes directed in the table below and are transferred in suitable amounts to an infusion or decoction apparatus.

Leaves, flowers and whole plants	Coarse cutting
Woods, stems, barks, roots and rhizomes	Moderately fine cutting
Seeds and fruits	Fine cutting

Infusion – Soak 50 g of herbal drugs into 50 mL of water for 15 min. Pour 900 mL of hot water and heat for 5 min with constant stirring. Filter through a cloth after cooling.

Decoction – Heat one day's dose of herbal drugs to 400 – 600 mL of water and boil for more than 30 min until half the amount of water added has been evaporated. Filter through a cloth while warm.

E. **Teabags** are preparations of one day or one dose of herbal drugs, cut into a size between coarse powder and coarse cutting and packed in paper or cloth bags. They are used according to the preparation method as directed under Infusion and Decoction.

F. **Tinctures** are liquid preparations obtained using 1 part herbal drugs and 10 parts of extraction solvent (ethanol or with a mixture of ethanol and purified water) or 1 part herbal drugs and 5 parts extraction solvent by means of maceration or percolation.

METHODS OF EXTRACTION

i. **Maceration** - commonly used method where the crude material being extracted is reduced to pieces of suitable size, if necessary, mixed thoroughly with a portion of the specified extraction solvent for about 15 min. The mixture is transferred to a percolator where a sufficient amount of the specified solvent is added to cover the entire solid mass, and the mixture is allowed to percolate slowly (at a rate of not more than 1 mL per minute for 1000 g of material). The matter to be extracted should always be covered with a layer of solvent. The residue may be pressed, and the obtained fluid is combined with the percolate. The entire percolates are concentrated, generally by distillation under reduced pressure, so as to subject the constituents of interest in the article under extraction to as little heat as possible.

ii. **Percolation** - the crude material being extracted is reduced to pieces of suitable size, mixed thoroughly with the specified extracting solvent, and allowed to stand at room temperature in a closed container for an appropriate time, with frequent agitation until the soluble matter is dissolved. The mixture is filtered, the insoluble material is washed with the same solvent used for maceration, and the filtrates are combined and concentrated, usually under reduced pressure, to the desired consistency.

G. **Soft Extracts** are semi-solid preparations obtained by evaporation or partial evaporation of the solvent used for extraction.

H. **Aromatic Waters** are clear liquid preparations, saturated essential oils or other volatile substances in water. They are prepared using the following process:

Shake thoroughly 2 mL of an essential oil or 2 g of a volatile substance with 1000 mL of lukewarm purified water. Shake for 15 min. Set the mixture aside for 12 hours or longer, filter through moistened filter paper, and add purified water to make 1000 mL. Alternatively, incorporate thoroughly 2 mL of an essential oil or 2 g of a volatile substance with sufficient talc, refined siliceous earth or pulped filter paper, add 1000 mL of purified water, agitate thoroughly for 10 min, and then filter the mixture. To obtain a clear filtrate, repeat the filtration if necessary, and add sufficient purified water that would pass through the filter paper to make 1000 mL.

FINISHED HERBAL PRODUCTS

Finished herbal products consist of herbal preparations made from one or more herbs. If more than one herb is used, the term "mixture herbal product" can also be used. Finished herbal products and mixture herbal products may contain excipients in addition to the active ingredients. However, finished herbal products or mixture herbal products to which chemically defined active substances have been added, including synthetic compounds and/or isolated constituents from herbal materials, are not considered to be herbal. Finished herbal products are available in dosage forms like capsules, tablets, suspensions, and ointments.

Capsules are solid dosage forms where the drug is enclosed within either a hard or soft soluble container or shell. Hard gelatin capsule shells are usually formed from gelatin. However, they may also be made from starch or other suitable substances. Surface active agents, opaque fillers, and antimicrobial preservatives may also be added to the capsule shell. The active substance(s), usually in solid form (as powdered plant part or dried, liquid, or semi-solid extract), is filled into the body of the capsule which is then closed by slipping the cap over it.

Herbal tablets are solid preparations intended for oral administration manufactured through compression. Herbal tablets contain active ingredients in the form of powdered plant part/s or extract and added excipients.

Suspensions are liquid dosage forms of active substance(s) (e.g. the dried, liquid, or semi-solid herbal extract) suspended finely and homogeneously in a vehicle, intended for oral administration. Suspensions are usually prepared by adding suspending agent or other suitable excipients and purified water or oil to the solid active substance(s), and suspending them homogeneously using a suitable method.

Ointments are semi-solid dosage forms intended for cutaneous or transdermal delivery of active ingredient/s or for its protective or emollient action. They may contain the solid or liquid drug dissolved or dispersed in a water-soluble, hydrophilic, or hydrophobic ointment base.

General Statements

QUALITY SYSTEMS

The **Philippine Herbal Pharmacopeia (PHP2022)** is the official book of standards and reference for the determination of the identity, purity, and quality of herbal drug products, traditional herbal medicines and crude plant drugs. This is an official publication, regarded as one of the important documents in ensuring and controlling the quality of medicinal plants and products. It will serve as a guide to the herbal pharmaceutical industry in terms of manufacturing, quality control, marketing and other standards of quality.

PHP2022 follows the initiative set forth by the Philippine Pharmacopeia I (PPI) in terms of setting standards including biological origins, descriptions of preparations and analytical procedures for testing and establishing quality and purity. The main focus of PHP2022 is on medicinal plants and drug products including those used as traditional herbal medicines. It also introduces new and emerging herbal preparations which are now considered important as alternative, complementary or integrative medicine in medical practice and in health care. It also aims to make quality improvements in the application of new technology instrumentation and qualitative methods in the analyses of these products. It allows for the internationalization of tests and measures using mainstream harmonized methods. Most of all, it updates and redirects the medicinal plant manufacturing industry to the emerging international standards and regulations on the manufacturing, quality control, marketing and regulation of medicinal plants, products and preparations.

The Philippine Herbal Pharmacopeia consists of two parts. Part 1 is on General Considerations and Part 2 is on Monographs. Since there is a growing demand for medicinal products and herbal plant preparations, there is a need to assure their quality and there must be sets of guidelines to follow in manufacturing to ensure safety, quality and efficacy. It is therefore essential that even the quality of the medicinal plants is being controlled like what's being done with chemically synthesized medicines.

Standardization, phytochemical investigation and various quality control methods are used to assure the quality of medicinal plant preparations. There are qualitative and quantitative methodologies that are employed for quality control assessment. Different techniques like UV/Vis and IR spectroscopy are mostly used for the qualitative aspects while HPTLC (High-Performance Thin-Layer Chromatography), HPLC (High-Pressure/Performance Liquid Chromatography), LC-MS (Liquid Chromatography-Mass Spectroscopy), AAS (Atomic Absorption Spectroscopy), and GC-MS (Gas Chromatography-Mass Spectroscopy) are used for the quantitative estimation. The Philippine Herbal Pharmacopeia serves to direct quality control measures to ensure product safety, quality and efficacy.

The PHP2022 is adapted from the PPI and other official Pharmacopeia in other countries such as the United States Pharmacopeia, International Pharmacopoeia, European Pharmacopeia, British Pharmacopeia, and the Japanese Pharmacopeia.

This Pharmacopeia also serves as a guideline for pharmaceutical manufacturers who would like to venture into herbal products processing and manufacturing.

STABILITY OF HERBAL PHARMACEUTICAL PRODUCTS AND TRADITIONAL MEDICINES

This guideline is based on the ASEAN Guidelines on Stability Study and Shelf Life of Traditional Medicines (Annex V adopted at the 20th ASEAN Traditional Medicines and Health Supplements Scientific Committee Meeting, August 26-29, 2013, Bangkok, Thailand). The guideline aims to provide recommendations on the core stability study required for products. It leaves sufficient flexibility to encompass the variety of different practical situations that may be encountered due to specific scientific considerations and characteristics of the products being evaluated. Although this guideline is yet to be adopted, the technical merits of the document already meet the needs of the Pharmacopeia.

The aim of conducting a stability test on medicinal plants and traditional medicines is to determine their shelf life in their pharmaceutical form, as a finished or prepared product in its final sealed container under the recommended storage condition. The product is required to meet its established physical, microbiological and/or chemical specifications within the expiry date stated.

STABILITY STUDY DESIGN AS PRESCRIBED BY THE ASEAN GUIDELINES ON STABILITY STUDY AND SHELF LIFE OF TRADITIONAL MEDICINE

GENERAL

The design of a stability study for the product should be based on the nature of the product. It should take into account the following:

- Selection of batches;
- Specifications/Testing parameters;
- Testing frequency
- Storage condition and
- Container closure system

SELECTION OF BATCHES

Stability data should be provided for batches of the same formulation and dosage form in the container closure system intended for marketing. Stability data from at least two batches are required, derived either from pilot scale, primary scale, production scale or their combination.

- The manufacturing process of batches used in stability studies should simulate that of production batches and should be of the same quality as well as meet the same specification as those batches intended for marketing.
- Stability studies, as discussed in ***Appendix 1***, should be performed on individual strengths of the product and/or the type of container closure system used for packing the finished product, unless bracketing/matrixing is applied.

SPECIFICATION AND TESTING PARAMETERS

A stability study should cover the testing of the physical, chemical, and microbiological properties of a finished product that are susceptible to change during storage and are likely to influence quality when changed. The list of testing parameters in ***Appendix 2*** serves as a guide for the types of tests to be included in a stability study.

The list of tests for each product is not meant to be exhaustive, nor is it expected to include every listed test used for the design of the stability study protocol for a particular finished product. For a product thats contains ingredients without known marker(s), physical parameters may be used as surrogate indicators during storage, when the use of such parameters can be justified. The physical parameters of the finished product can be checked by at least one of the following test methods:

I. Gross organoleptic analysis; Examination by general impression
II. Other scientifically valid criteria.

Although it may not be necessary to assay all the active ingredients, it may be appropriate for a combination product containing multiple active ingredients to assay one, and in some cases, more than one active ingredient, or a surrogate

marker known to be susceptible to change during storage and is likely to influence the quality of the combination product. A valid justification should be submitted.

TESTING FREQUENCY

For accelerated and real-time stability studies, the frequency of testing should be sufficient to establish the stability profile of the finished product. At the accelerated storage condition, a minimum of three time points, including the initial and final time points, for example, 0, 3, and 6 months for a 6-month study, is recommended. The frequency of testing at real-time storage conditions should normally be every 3 months over the first year, every 6 months over the second year and annually thereafter throughout the proposed shelf life. A typical testing frequency is shown in ***Table 2.8*** below.

Table 2.8. A Typical Testing Frequency

STORAGE CONDITION	TESTING FREQUENCY
Real-time	0, 3, 6, 9, 12, 18, 24 months and annually thereafter throughout the proposed shelf life
Accelerated	0, 3 and 6 months

Real-time and accelerated studies/testing are undertaken on primary batches according to a prescribed stability protocol to establish or confirm the re-test period of a substance or shelf life of a finished product.

- **Accelerated Stability Studies**

  These are studies designed to increase the rate of chemical degradation or physical change of a finished product by using exaggerated storage conditions as part of the formal stability studies. (Data from these studies, in addition to long-term stability studies, can be used to assess longer term chemical effects at non-accelerated conditions and to evaluate the effect of short-term excursions outside the label storage conditions such as those that may occur during shipping. Results from accelerated testing studies are not always predictive of physical changes).

- **Real-time Stability Studies**

  Stability studies under the recommended storage condition for the re-test period or shelf life proposed (or approved) for labeling.

Reduced designs, i.e., matrixing or bracketing, where the testing frequency is reduced or certain factor combinations are not tested at all, can be applied, as discussed in ***Appendix 1***. If, based on development experience, outcomes from accelerated studies are likely to approach significant change criteria (i.e., parameters tested are out of the specifications set), conduct an increased testing, either by adding samples at the final time point or by including a fourth time point in the study design. If the "significant change" occurs within the first 3 months of testing at the accelerated storage condition, a justification should be provided to address the effect of short-term excursions outside the label storage condition, e.g., during shipping or handling. This justification can be supported, if appropriate, by further testing on a single batch of the product for a period shorter than 3 months but with more frequent testing than usual. It is considered unnecessary to continue to test a product through the remaining months when a "significant change" has occurred within the first 3 months, and as such, the shelf life will be based on real-time data. This can be applied to products such as ointments, creams, or suppositories that are impossible to test at accelerated condition whereby only real-time testing is required. If "significant change" occurs between 3 and 6 months' testing at the accelerated storage condition, shelf life will be based on real-time data.

STORAGE CONDITION

In general, Traditional Medicine (TM) as a finished product should be evaluated under its storage conditions (with appropriate tolerances) that test its thermal stability under recommended storage conditions and, if applicable, its sensitivity to moisture or potential for solvent loss. The storage conditions and the lengths of studies chosen should be sufficient to cover the storage, shipment, and subsequent use, for example, after reconstitution or dilution as recommended in the labeling. Specific recommended temperature and relative humidity conditions for storage applied to stability studies

and types of container closure system are based on the nature of the products and the type of primary container used, in accordance with the recommended storage condition on product label. Common storage conditions are shown in ***Table 2.9*** below.

Table 2.9. Common Storage Conditions

TYPE OF CONTAINER CLOSURE SYSTEM / STUDY	STORAGE CONDITION
Products in primary containers permeable to water vapor	30°C ± 2°C/75% RH ± 5% RH
Products in primary containers impermeable to water vapor	30°C ± 2°C
Accelerated studies	40°C ± 2°C/75% RH ± 5% RH

If submitted data are based on conditions that are less stressful (e.g., 30°C/65% RH) than those required, the data should be accompanied by appropriate complementary data which will allow the conduct of a proper scientific evaluation. Factors to be taken into consideration include:

- Whether any instability is seen;
- Whether data have also been provided under accelerated conditions;
- The type of container closure system

Other storage conditions are allowable, if justified. Examples include:

- Heat-sensitive products which should be stored under lower temperature conditions, which would eventually become the designated long-term storage temperature.
- Products containing less stable active ingredients and formulations not suitable for storage at elevated temperature (e.g., suppositories) will need real-time stability studies.
- If a lower temperature condition is used, the 6-month accelerated testing should be carried out temperature of at least 15°C above the expected actual storage temperature (together with appropriate relative humidity conditions for that temperature). For example, for a product to be stored long-term under refrigerated conditions, accelerated testing should be conducted at 25ºC ± 2ºC, 60% RH ± 5% RH. The designated real-time testing conditions will be reflected in the labeling and shelf life (expiration date). Typical storage conditions recommended for stability studies on products intended for storage in a refrigerator is shown in ***Table 2.10*** below.

  Table 2.10. Typical Storage Conditions Recommended for Products Intended in a Refrigerator

STUDY	STORAGE CONDITION
Real-time	5°C ± 3°C
Accelerated	25°C ± 2°C/60% RH ± 5% RH

- Products which change physically or even chemically at lower storage temperature conditions e.g., suspensions or emulsions, which may sediment or cream; oils and semi-solid preparations, which may show an increased viscosity.

Data from the accelerated stability studies can be used to evaluate the effect of short-term excursions outside the label storage conditions such as during shipping. The data from accelerated study and ongoing real-time stability study can be used to justify an interim extrapolated shelf life. However, the actual shelf life should be based ultimately on the real-time stability data at the recommended storage conditions.

CONTAINER CLOSURE SYSTEM

Stability testing should be conducted on the product packaged in the primary container closure system proposed for marketing including, whenever appropriate, any secondary packaging.

Finished products packed in moisture-impermeable primary containers are not required to be tested under high humidity conditions. Generally considered moisture-impermeable containers include aluminum/aluminum blisters, High Density Polyethylene (HDPE) or glass bottles fitted with metal or HDPE closures. When using moisture-permeable containers for packaging, due consideration should be given to the stability of the contents under high humidity conditions. Moisture may have an undesirable effect on chemical and physical stability of a finished product.

The issue of the different permeability of various packaging materials should be addressed e.g., the effect of high humidity on solid dosage forms packaged in containers permeable to moisture should be supported by data and an indication, like "keep in a dry place or protect from moisture" should be added to the label. Examples of moisture permeable containers include polyvinyl chloride (PVC) blisters, low density polyethylene (LDPE) bottles, glass or HDPE bottles when fitted with polypropylene closures.

EVALUATION

A systematic approach should be adopted in the presentation and evaluation of the stability information, which should include, whenever appropriate, results from the physical, chemical and microbiological tests. Any evaluation should consider not only the assay, but also other appropriate test attributes. A recommended presentation of the summary table of stability results is provided in ***Appendix 3***.

LABELING

The storage conditions that include temperature, light and humidity indicated on the label should be based on the stability evaluation of the product. General precautionary statements, such as "Protect from light" and/or "Store in a dry place," may be included, but should not be used to conceal stability problems of the finished product. Specific instructions on storage conditions should be provided. Terms such as "ambient conditions" or "room temperature" should be avoided.

*Appendices at the end of this section.
**Available full document at: https://asean.org/wp-content/uploads/2017/09/ASEAN-Guidelines-on-Stability-and-Shelf-Life-HS-V1.0-with-disclaimer.pdf

GUIDELINES FOR VALIDATION OF ANALYTICAL PROCEDURES

Validation is defined in simple terms as the act of officially or legally certifying, approving or accepting a process. In the pharmaceutical industry, validation is the process of establishing documentary evidence demonstrating that a procedure, process, or activity carried out in testing and then production maintains the desired level of compliance in all stages. It is very important that, in addition to final testing and compliance of products, it is also assured that the process will consistently produce the expected results. The term "validation" is meant to apply to the final evaluation and verification of the manufacturing process. A minimum of three consecutive production batches should be successfully validated prior to the approval and marketing of the product.

In terms of the *Pharmacopeial Methods* used in this document, validation of methods are completed to ensure that an analytical methodology is accurate, specific, reproducible and rugged over the specified range that a target analyte will be analyzed. Assay validation provides an assurance of reliability during normal use, and is oftentimes referred to as "the process of providing documented evidence that the method does what it is intended to do."

Methods validation is the evaluation of a test method or an analytical method to determine its applicability for a particular analyte. In a validation process, the performance parameters of a method are obtained to verify that they are sufficient for providing the data to answer a particular problem or question for which the method is intended to be used.

Results from a method validation can be used to evaluate the quality, reliability and consistency of analytical results. It is therefore an integral part of any good and credible analytical practice. Its applicability to herbal preparations and products cannot be discounted to ensure consistency in quality.

ASSAY VALIDATION LEVELS AND STEPS

ASSAY OPTIMIZATION (PRE-VALIDATION)

Assay optimization and pre-validation are experiments that determine how a range of matrix and analyte characteristics, as well as assay conditions, will consistently affect the assay parameters and assay performance. These data, along with a sound scientific judgment, set the acceptance criteria, such as numerical limits, ranges, or other criteria for tests that are used for or in making a decision to accept or reject a unit, lot, or batch or a batch of a pharmaceutical product or preparation, for the assay validation. It is important to establish acceptance criteria before executing the validation protocol.

ASSAY QUALIFICATION

Qualification is an act or process that ensures that something complies with preset conditions, established standards, or specific requirements. Assay qualification is an experimental process intended to demonstrate that an accepted method will provide meaningful data for the specific conditions, matrix and samples that the procedure is intended for. Assay Qualification may not require validation of accuracy and reliability of the method (sensitivity), but merely verify the suitability of the protocol under actual conditions (generally, specificity) of the assay.

ASSAY VALIDATION

Validation, meanwhile, is a process of documenting objective evidence that provides a high degree of assurance that a specific process or method will consistently produce a product meeting its predetermined specifications. Comprehensive experiments that evaluate and document the quantitative performance of an assay, including sensitivity, specificity, accuracy, precision, detection limit, range and limits of quantitation. Full assay validation will include inter-assay and inter-laboratory assessment of assay repeatability and robustness.

DEFINITIONS OF THE ASSAY PARAMETERS

Specificity is the ability to assess without a doubt the target analyte in the presence of components which might be expected to be present. The specificity of an assay is the capability of the assay to differentiate similar analytes or other interference from matrix elements that could have a positive or negative effect on the assay value.

IDENTIFICATION: To ensure the identity of the target analyte.
ASSAY (Content or Potency): To provide an exact result which allows an accurate statement on the content or potency of the target analyte in a sample.

Accuracy is the agreement between the value found and an accepted reference value. This requires a "gold" standard or method but in the absence of a gold standard or method, comparison to established reference laboratories may substitute.

The accuracy of the analytical process is the measure expressing the similarity between the values found by the conventional analytical method (with proven accuracy) and the estimated values found using prescribed alternative methods that may involve new instrumentation. The accuracy should be proven in the whole range specified by the

analytical method. The accuracy is a parameter evaluated only after establishing precision, linearity and specificity. The accuracy can be evaluated by at least 9 measurements in the specified range. For example, the results are measured by manipulating 3 levels of concentration and 3 repetitions of each concentration level. Three minimum levels of concentrations are selected as the mean value and both of the maximum and the minimum. The accuracy is expressed as the recovery, when the samples spiked with a known level of the analyte are quantitated, or are examined by comparison between the reference value and the mean value of measurements in the confidence interval, when the comparison with the true value or the certified or agreed value is made. The calibration set should consist of the samples with the concentrations of the active ingredients produced in the batch process and it should include the whole range of the sample. The selected samples should show the uniform distribution of probability, not the normal distribution. The validation set should consist of the samples with the same composition of the active ingredients as in the calibration set. The set of samples for the parallel test are selected from the independent production batch and the analysis is validated by the time. Therefore, the accuracy of the analysis can be examined using the set completely independent from the calibration set and the validation set of samples. This test is performed once a month with independent set of samples after the development of the calibration model.

When measuring accuracy, it is important to spike placebo preparations with varying amounts of the active ingredient(s). If a placebo cannot be obtained, then a sample should be spiked at varying levels or standard addition method could be employed. In both cases, acceptable recovery must be demonstrated.

STANDARD ADDITION METHOD

Standard addition must be used whenever the matrix of a sample changes the analytical sensitivity of the method. It is a calibration method that involves adding an additional amount of the substance of interest to a sample when a one-off or infrequently performed analysis is required. An analyst usually divides the unknown sample into two portions, so that a known amount of the analyte (a spike) can be added to one portion. These two samples, the original and the original plus spike, are then analyzed. The sample with the spike will show a larger analytical response than the original sample due to the additional amount of analyte added to it.

PRECISION

The precision of an analytical method means there is similarity among the results obtained by a series of repeated tests with the same analyte under the specified analytical conditions. The items of precision are repeatability, within-laboratory reproducibility and inter-laboratory reproducibility. Precision is tested with homogeneous and authentic samples under the normal assay conditions. When the homogeneous and authentic samples are not easily available, the test can be done with artificially synthesized samples or solutions. The precision is expressed as the relative standard deviation or the coefficient of variation calculated from a series of measurements. For enzyme assays, precision is usually <10%; 20% to 50% for in vivo and cell based assays; and >300% for virus titer assays.

Precision includes within-assay variability, repeatability (within-day variability), and reproducibility (day-to-day variability). Precision may be established without the availability of a "gold" standard as it represents the scatter of the data rather than the exactness (accuracy) of the reported result. The precision of an analytical method is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements.

Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also called intra-assay precision.

Intermediate Precision expresses within-laboratory variations: different days, different analysts, different equipment, etc.

Reproducibility expresses the precision between laboratories (collaborative studies usually applied to standardization of methodology).

The representative factors of variation which need examination in within-laboratory reproducibility are the date of experiment, the analyst, the instrument used and the condition of the assay. The inter-laboratory reproducibility is evaluated when the analytical method is needed to be standardized for the collaborative experiments between laboratories.

If the inter-laboratory reproducibility is proven, the within-laboratory reproducibility need not be validated, but usually, the within-laboratory reproducibility is the one mainly evaluated because the inter-laboratory reproducibility is hard to achieve.

Detection Limit is the lowest amount of analyte that can be detected, but not necessarily quantified as an exact value. The detection limit is a low concentration that is statistically distinguishable from background or negative control, but is not sufficiently precise or accurate to be quantified.

Limits of Quantitation are the lowest and highest concentrations of an analyte in a sample that can be quantitatively determined with suitable precision and accuracy. The lower limit of quantitation is often defined by an arbitrary cut-off such as a ratio of signal-to-noise, equal to 1:10, or a value equal to the mean of the negative control plus 5 times the standard deviation of the negative control values.

Linearity is the ability of the assay to return values that are directly proportional to the concentration of the target analyte in the sample. Mathematical data transformations, to promote linearity, may be allowed if there is scientific evidence that the transformation is appropriate for the method. The range is the concentration of analyte or assay values between the low and high limits of quantification. Within the assay range, linearity, accuracy, and precision are acceptable.

Classical linearity acceptance criteria require:

- the correlation coefficient of the linear regression line to be not more than some number close to 1
- the y-intercept to not differ significantly from zero.

When linear regression analyses are performed, it is important not to force the origin as (0,0) in the calculation. This practice may significantly skew the actual best-fit slope through the physical range of use.

Range is the interval between the maximum and the minimum of concentrations used for measuring linearity, precision and accuracy during the analytical process. It is calculated by subtracting the lowest value from the highest value. While a high range means high variability, a small range means low variability in a distribution.

The formula to calculate the range is:

$$R = H - L$$

- R = range
- H = highest value
- L = lowest value

The range is the easiest measure of variability to calculate. To find the range, follow these steps:

1. Order all values in your data set from low to high.
2. Subtract the lowest value from the highest value.

This process is the same regardless of whether your values are positive or negative, or whole numbers or fractions.

Ruggedness is the reproducibility of the assay under a variety of normal, but variable, test conditions. Variable conditions might include different equipment, analysts or operators, and reagents. Ruggedness provides an estimate of experimental reproducibility with unavoidable error. It is normally expressed as the lack of influence on the test results of operational and environmental variables of the analytical method.

Robustness is a measure of the assay capacity to remain unaffected by small but deliberate changes in test conditions. Robustness provides an indication of the ability of the assay to perform under normal usage. Robustness measures the effect of deliberate changes (temperature, sample preparation, buffer pH) that can be controlled through specifications in the assay protocol.

Note: Ideally, robustness should be explored during the development of the assay method. By far the most efficient way to do this is through the use of a designed experiment. Such experimental designs may include:

- a Plackett-Burman matrix approach to investigate first-order effects, or
- a 2k factorial design that will provide information regarding the first (main) and higher order (interaction effects).

SYSTEM SUITABILITY

In addition, prior to the start of laboratory studies to demonstrate method validity, some type of system suitability must be done to demonstrate that the analytical system is performing properly and is suitable for the required analyses.

For this Pharmacopeia examples may include but not limited to:

a. replicate injections of a standard preparation for HPTLC, HPLC and other methods;
b. standardization of a volumetric solution, followed by assays using the same burette for titrimetric methods;
c. replicate scanning of the same standard preparation during UV-VIS assays, etc.

When the method in question utilizes an automated system such as a chromatograph or an atomic absorption spectrophotometer, a suitable standard preparation (or a suitable common marker) should be intermittently measured during the sample analysis run.

The responses generated by the standard should exhibit a reasonable relative standard deviation. This is done primarily to demonstrate the stability of the system during sample measurements.

PROTOCOLS

Prior to initiating a validation study, a well-planned validation protocol should be written and reviewed for scientific soundness and completeness by qualified individuals. The protocol should describe the procedure in detail, and should include predefined acceptance criteria and predefined statistical methods. Following the approval of the appropriate "Quality Control" authorities, the protocol should be executed in a timely manner. A typical assay validation will require the preparation of product placebo(s), standards, and many replicate samples. The assay should be repeated to ensure "validity"; three (3) sequential replicates are often considered the "magic number." However, a far more definitive number is one produced by a sound scientific rationale, usually with the assistance of statistical analyses. Subsequent to the execution of the protocol, the data must be analyzed with results, conclusions and deviations presented in an official validation summary report. Provided that the predefined acceptance criteria are met, and the deviations (if any) do not affect the scientific interpretation of the data, the method can be considered valid. A statement of the method's validity should be placed at the beginning of the final summary report, along with the signatures and titles of all significant participants and reviewers. https://www.pharmaguideline.com/2011/08/analytical-method-validation-with.html

For these reasons, **method validation and accreditation** are essential requirements of accreditation to ISO/IEC 17025

NORMATIVE REFERENCES

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

- **ISO/IEC 17000:2020,** Conformity Assessment — Vocabulary and General Principles
- **ISO/IEC 17025:2017,** General Requirements for the Competence of Testing and Calibration Laboratories
- **ISO 9001:2015,** Quality Management
- **ISO/IEC Guide 2:2004,** Standardization and Related Activities — General Vocabulary
- **ISO/IEC** Guide **99:2007**, International Vocabulary of Metrology — Basic and General Concepts and Associated Terms

ISO/IEC 17025 enables laboratories to demonstrate that they operate competently and generate valid results, thereby promoting confidence in their work both nationally and around the world. It also helps facilitate cooperation between laboratories and other bodies by generating wider acceptance of results between countries. Test reports and certificates can be accepted from one country to another without the need for further testing, which, in turn, improves international trade.

The ISO 17025 accreditation is the most important standard for testing and/or calibration laboratories. It confirms that a particular laboratory is able to produce precise and accurate test results and calibration data, including: traceability of measurements and calibrations to national standards.
Link to **ISO 9001:** https://www.iso.org/iso-9001-quality-management.html

ISO 9001: The standard used by organizations to demonstrate their ability to consistently provide products and services that meet customer and regulatory requirements and to demonstrate continuous improvement.
Links to **ISO/IEC Standards:** https://www.iso.org

REFERENCE FOR REGULATORY DOCUMENTS

1. **The Philippine Constitution 1987:**
   a. Article II, Section 15: to "protect and promote the right to health of the people and instill health consciousness among them" and;
   b. Article XIII, Section 12: that "The State shall establish and maintain an effective food and drug regulatory system and undertake appropriate health manpower development and research, responsive to the country's health needs and problems".

   Cognizant of these, a national pharmacopeia establishes standards for drug/drug products and crude plant drugs available in commerce in the Philippines. This Pharmacopeia is essential to the attainment of an effective regulatory and health care delivery systems. (adapted from Philippine Pharmacopeia 1, 2004)
   Full text of the 1987 Philippine Constitution at https://www.officialgazette.gov.ph/constitutions/

2. **Presidential Executive Order No. 302** declaring and adopting the Philippine Pharmacopeia as the official book of standards and reference for Pharmaceutical Products and Crude Drugs For the **Philippine Pharmacopeia (PP) 1st edition April 2004**
   https://www.officialgazette.gov.ph/2004/03/29/executive-order-no-302-s-2004/

3. **FDA AO 172 Series of 2004: Guidelines on the Registration of Herbal Medicine**
   https://www.fda.gov.ph/wp-content/uploads/2021/04/Administrative-Order-No.-172-s.-2004.pdf

4. **FDA AO 184 Series of 2004: Guidelines on the Registration of Traditionally Used Herbal Products**
   https://www.fda.gov.ph/wp-content/uploads/2021/04/Administrative-Order-No.-184-s.-2004.pdf

5. **Center for Drug Regulation and Research: List of Requirements for Registration of Herbal Medicines**
https://www.fda.gov.ph/wp-content/uploads/2021/05/Initial-Registration-of-Herbal-Medicines.pdf

6. **International Conference on Harmonization (ICH)**
International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
Test Procedures and Acceptance Criteria for New Drug Substances and New Drug Products:
https://database.ich.org/sites/default/files/Q6A_Guideline.pdf

7. **WHO Guidelines on Quality Control of Herbal Medicines**
World Health Organization. (2007). WHO guidelines for assessing quality of herbal medicines with reference to contaminants and residues.
https://apps.who.int/iris/handle/10665/43510

8. **WHO Guidelines on Good Manufacturing Practices (GMP) for Herbal Medicines**
World Health Organization. (2007).
https://apps.who.int/iris/handle/10665/43672

9. **Republic Act No. 8423: Traditional and Alternative Medicine Act of 1997**
An act creating the Philippine Institute of Traditional and Alternative Health Care (PITAHC) to accelerate the development of traditional and alternative health care in the Philippines, providing for a traditional and alternative health care development fund and for other purposes.

10. **Pharmaceutical Inspection Convention/ Pharmaceutical Inspection Cooperation Scheme PIC/S GMP Guidelines PE 009-16 (Annexes) 1 February 2022**
Annex 7: Manufacture of Herbal Medicinal Plants
https://picscheme.org/docview/4590

PHARMACOPEIAL HARMONIZATION

Internationalization and expansion in trade present a growing need to develop global quality standards for herbal medicines and crude plant drugs. Standards are vital to marketing authorizations, market surveillance, and free movement and trade of herbal products and crude plant drugs between regions and countries.

The Philippine Herbal Pharmacopeia make references to the latest editions of the United States Pharmacopeia/National Formulary (USP/NF), Japanese Pharmacopeia (JP), British Pharmacopeia (BP), European Pharmacopeia (EP), International Pharmacopeia (Ph. Int.), emerging Pharmacopeia in the ASEAN region and other books of standards and references like those of the International Council on Harmonization and ASEAN Harmonization that are recognized by the Philippine Food and Drug Administration from time to time.

The European Pharmacopeia is engaged in a process of harmonization with the European, Japanese and United States Pharmacopeia, within an informal structure referred to as the Pharmacopeial Discussion Group (PDG). The PDG was formed in 1989 with participants from the European Directorate for the Quality of Medicines in the Council of Europe (the European Pharmacopoeia), the Ministry of Health, Labor, and Welfare (MHLW) for the Japanese Pharmacopoeia, and the United States Pharmacopeial Convention, Inc. (for the U.S. Pharmacopeia). In May 2001, PDG welcomed the World Health Organization as an observer. PDG generally meets twice a year and holds monthly status and technical teleconferences to advance harmonization work. USP participates in the PDG to work on pharmacopeial harmonization topics.

The Pharmacopeial Discussion Group (PDG), consisting of the European Pharmacopoeia (EP), Japanese Pharmacopoeia (JP), and the United States Pharmacopoeia (USP), has agreed to the implementation of significant changes to the work structure, including:

- Eliminating two stages of PDG harmonization process to streamline and reduce the level of complexity.
- Restructuring meeting format to engage more effectively at the technical level with regards to resolving issues on specific topics.
- Maintaining a twice-yearly meeting frequency with a focus on strategic direction setting.

Effective April 1, 2018, PDG utilizes a reduced 5-stage approach for harmonization of retrospectively for existing monographs or chapters or prospectively for new monographs or chapters.

In this new structure shown in ***Table 2.11***, Stage 1 and Stage 2 will be officially removed and handled more informally within PDG. The previous stages 4, 5 and 6 are now labeled 2, 3 and 4.

Table 2.11. Current and New PDG Stage Structure

PREVIOUS STRUCTURE	CURRENT (EFFECTIVE APRIL 1, 2018)
Stage 1: Identification	Stage 1: Preparation of First Draft
Stage 2: Investigation	
Stage 3: Proposal for Expert Committee Review	
Stage 4: Official Inquiry	**Stage 2: Official Inquiry**
Stage 5: Consensus	Stage 3: Consensus
Stage 6: Regional Adoption and Implementation	**Stage 4: Regional Adoption and Implementation**
Stage 7: Inter-Regional Acceptance	Stage 5: Inter-Regional Acceptance

This is the link to the Statement of Harmonization Policy of PDG: https://www.usp.org/harmonized-standards/pdg

"Where harmonization of general chapters is carried out, the aim is to arrive at interchangeable methods or requirements so that demonstration of compliance using a general chapter from one of the 3 pharmacopeias implies that the same result would be obtained using the general chapter of either of the other pharmacopeias. When a formal declaration of interchangeability has been recommended by the International Conference on Harmonisation (ICH), it will be indicated in general chapters of these pharmacopeias: "in the process of Pharmacopeial harmonization." If residual differences remain in harmonized general chapters, information is given in the general chapter. Where harmonisation of monographs is carried out, the aim is to arrive at identical requirements for all attributes of a product."

Besides the PDG, the European Pharmacopeia is also actively involved in a number of other international harmonization initiatives, such as the World Health Organization initiative of drafting "Good Pharmacopeial Practices" that may serve as basis for future work-sharing and collaboration between the pharmacopeias of the world.

In this context, the Philippine Herbal Pharmacopeia also aims to establish such harmonization with existing ASEAN and other international pharmacopeias to better establish international standards of quality, purity and efficacy of related products. ASEAN Guidelines are consulted whenever necessary and adopted for harmonization of procedures to ensure the quality, safety and efficacy of herbal products and preparations.

APPENDICES

APPENDIX 1
STABILITY STUDY: REDUCED DESIGN (BRACKETING AND MATRIXING)

A full study design is one in which samples for every combination of all design factors are tested at all time points whereas a reduced design is one in which samples for every factor combination are not all tested at all time points. A reduced design can be a suitable alternative to a full design when multiple design factors are involved. Any reduced design should have the ability to adequately predict the shelf life. Before a reduced design is considered, certain assumptions should be assessed and justified. The potential risk when establishing a shorter shelf life using a reduced design should be considered due to the reduced amount of data collected compared to data derived from a full design.

During the course of a reduced design study, a change to full testing or to a less reduced design can be considered if justification is provided and the principles of full designs and reduced design studies are followed. However, proper adjustments should be made to the statistical analysis, where applicable, to account for the increase in sample size as a result of the change. Once the design is changed, full testing or less reduced testing should be carried out through the remaining time points of the stability study.

BRACKETING

Bracketing is the design of a stability schedule such that only samples on the extremes of certain design factors, for example, strength, container size and/or fill, are tested at all time points as in a full design. The design assumes that the stability of any intermediate levels is represented by the stability of the extremes tested.

DESIGN EXAMPLE

An example of a bracketing design is given in ***Appendix Table 2.1***. This example is based on a product available in three strengths and three container sizes. In this example, it demonstrated that the 15 mL and 500 mL high-density polyethylene container sizes truly represent the extremes. The batches for each selected combination should be tested at each time point as in a full design.

Appendix Table 2.1. Example of Bracketing Design

Strength		50 mg			75 mg			100 mg		
Batch		1	2	3	1	2	3	1	2	3
Container Size	15 mL	T	T	T				T	T	T
	100 mL									
	500 mL	T	T	T				T	T	T

Key: T = Sample tested

MATRIXING

Matrixing is the design of a stability schedule such that a selected subset of the total number of possible samples for all factor combinations would be tested at a specified time point. At a subsequent time point, another subset of samples for all factor combinations would be tested. The design assumes that the stability of each subset of samples tested represents the stability of all samples at a given time point. The differences in the samples for the same finished product should be identified as, for example, covering different batches, different strengths, different sizes of the same container closure system, and possibly, in some cases, different container closure systems. When a secondary packaging system contributes to the stability of the finished product, matrixing can be performed across the packaging systems. Each storage condition should be treated separately under its own matrixing design. Matrixing should not be performed across test attributes. However, alternative matrixing designs for different test attributes can be applied if justified.

DESIGN EXAMPLES

Examples of matrixing designs on time points for a product in two strengths (S1 and S2) are shown in ***Appendix Table 2.2*** below. The terms "one-half reduction" and "one-third reduction" refer to the reduction strategy initially applied to the full study design. For example, a "one-half reduction" initially eliminates one in every two time points from the full study design and a "one-third reduction" initially removes one in every three. In the examples shown in ***Appendix Table 2.2***, the reductions are less than one-third due to the inclusion of full testing of all factor combinations at some time points. These examples include full testing at the initial, final, and 12-month time points.

Appendix Table 2.2. Example of Matrix Design on Time Points for a Product with Strengths "One-Half Reduction"

Time point (months)			0	3	6	9	12	18	24	36
Strength	S1 S2	Batch 1	T	T		T	T		T	T
		Batch 2	T		T		T	T		T
	S2 S2	Batch 1	T		T		T		T	T
		Batch 2	T	T		T	T	T		T
				At least 6 out of 12 time points				At least 4 out of 8 time points		

Time point (months)			0	3	6	9	12	18	24	36
Strength	S1 S2	Batch 1	T	T		T	T	T	T	T
		Batch 2	T	T	T		T	T		T
	S2 S2	Batch 1	T		T	T	T		T	T
		Batch 2	T	T		T	T	T	T	T
				At least 8 out of 12 time points				At least 6 out of 8 time points		

Time point (months)			0	3	6	9	12	18	24	36
Strength	S1 S2	Batch 1	T	T		T	T	T	T	T
		Batch 2	T	T	T		T	T		T
	S2 S2	Batch 1	T		T	T	T	T		T
		Batch 2	T	T		T	T		T	T
	S3 S3	Batch 1	T		T	T	T	T	T	T
		Batch 2	T	T		T	T		T	T
				At least 12 out of 18 time points				At least 8 out of 12 time points		

Key: T = Sample tested

APPENDIX 2
TABULATED LIST OF STABILITY INDICATING PARAMETERS FOR HEALTH SUPPLEMENT AND TRADITIONAL MEDICINES

The tabulated list of parameters for each dosage form is presented as a guide for the following types of tests to be included in a stability study.

Appendix Table 2.3. HS Dosage Form

Testing Parameters / HS Dosage Form	Organoleptic Characteristics	Assay	Hardness/Friability	Dissolution/Disintegration	Water Content	Viscosity	pH	Microbial Content	Granules/Particles Size	Resuspendability
Oral powder	✓	✓			✓			✓		
Hard capsule	✓	✓		✓	✓			✓		
Soft capsule	✓	✓		✓				✓		
Coated and Uncoated Tablet	✓	✓	✓	✓	✓			✓		
Coated and Uncoated Pill/Pellet	✓	✓		✓	✓			✓		
Suspension	✓	✓				✓	✓	✓	✓	✓
Solution	✓	✓				✓	✓	✓		
Emulsion	✓	✓				✓	✓	✓		
Granules	✓	✓						✓	✓	

Appendix Table 2.4. TM Dosage Form

Testing Parameters / TM Dosage Form	Organoleptic Characteristics	Assay	Hardness/Friability	Dissolution/Disintegration	Water Content	Viscosity	pH	Microbial Content	Granules/Particles Size	Resuspendability	Adhesiveness
Oral powder	✓	✓			✓			✓			
Hard capsule	✓	✓		✓	✓			✓			
Soft capsule	✓	✓		✓				✓			
Coated and Uncoated Tablet	✓	✓	✓	✓	✓			✓			
Coated and Uncoated Pill/Pellet	✓	✓		✓	✓			✓			
Suspension	✓	✓				✓	✓	✓	✓	✓	
Solution	✓	✓				✓	✓	✓			
Emulsion	✓	✓				✓	✓	✓			
Semi-Solid Preparations (Ointment/Cream/ Gel/Lotion/Paste)	✓	✓				✓	✓	✓			
Plaster	✓	✓						✓			✓
Granules	✓	✓			✓			✓	✓		
Herbal Infusion Bag or Herbal Tea Bag	✓	✓			✓			✓			
Pastilles	✓	✓			✓			✓			

APPENDIX 3
RECOMMENDED PRESENTATION OF THE SUMMARY TABLE OF STABILITY RESULTS

Product Name : _____ **Storage Condition** : _____

Dosage Form : _____ **Batch No.** : _____

Strength : _____ **Manufacturing Date** : _____

Container : _____ **Date of Report** : _____

Packaging Size : _____ **Period of the Study** : _____

Testing Parameters (as applicable)	Permissible Level/Acceptance Criteria Characteristics	Testing Frequency (Months)							
		0	3	6	9	12	18	24	
Oroganoleptic characteristics									
Assay									
Hardness/Friability									
Dissolution/Disintegration									
Water Content									
Viscosity									
pH									
Microbial Content									
Granules/Particle Size Variation									
Resuspendability									

Conclusion :

Prepared by **Checked by** **Approved by**

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General Chapters | General Methods

I. Test for Crude Plant Drugs

<1> SAMPLING

The following sampling procedures are the minimum considered applicable to crude plant drugs. Some articles or some tests may require more meticulous procedures involving more containers being sampled or more samples per container. In this chapter, proper handling to obtain gross, laboratory and test samples are discussed.

GROSS SAMPLE

Take individual samples from the number of randomly selected containers and make sure that upon examination, the container markings and labels indicate that the batch can be considered homogenous. Refer to ***Table 3.1***

Table 3.1. Gross Sampling Scheme

NUMBER OF CONTAINERS	
Batch Size (N)	For Sampling (n)
1-10	All
11-19	11
>19	*n=10 + (N/10)

*Calculated ***n*** is rounded to the next highest whole number when applicable

For batches that cannot be considered homogenous, divide it into sub-batches that are as homogenous as possible and sample each one as a homogenous batch.

Take samples from the upper, middle and lower sections of each container. For plant material consisting of component parts which are 1 cm or less in any dimension, as well as all powdered or ground materials, withdraw the sample by means of a sampling device that removes a core from the top to the bottom of the container, not less than two cores being taken in from opposite directions. Samples may be withdrawn by hand for materials with component parts of more than 1 cm in any dimension.

In case of large-scale bales or packs, samples should be taken from a depth of 10 cm as the moisture content of the surface layer may be different from the inner layer.

Prepare the gross samples by combining and mixing the individual samples taken from each opened container, making sure not to increase the fragmentation or significantly affect the moisture content.

PROCESSING OF TEST SAMPLE

Test specimens should be processed before testing, unless otherwise directed in the individual monograph or test procedure. Depending on the required quantity for determination, divide the sample herbs into four small portions by quartering, taking care that each withdrawn portion remains representative. For unground or unpowdered crude plant drugs, grind the withdrawn sample and let it pass through a No. 20 mesh sieve (850 µm). Mix the resulting powder well. If the material cannot be ground, reduce it to a fine state if possible, mix by rolling it on paper or sampling cloth. Spread it out in a thin layer and withdraw the portion for analysis by quartering. Then prepare for test determination.

TEST SAMPLE FOR FOREIGN MATTER

Table 3.2. Test Sample

Roots, rhizomes, bark and herbs	500 g
Leaves, flowers, seeds and fruit	250 g
Cut crude plant drugs (average mass of the pieces is less than 0.5 g)	50 g

<2> FOREIGN MATTER

Spread out the sample in a thin layer, and separate the foreign matter (such as stem fragments in leaf drugs, other plants, insect parts, sand, and etc.) as completely as possible. Inspect either with unaided eye or with the use of a magnifying glass of 10 magnifications. Weigh and determine the percentage of foreign matter, using the formula:

$$Foreign\ matter\ (\%) = \frac{M_f}{M_s} \times 100$$

where:

- M_f = mass of foreign matter in gram found in the "test sample for foreign matter" taken;
- M_s = is the mass of "test sample for foreign matter" taken.

<3> DETERMINATION OF WATER CONTENT

PREPARATION OF TEST SAMPLES

Take 10 g of "test sample." If the test sample is not finely ground, grind it until the thickness of the parts does not exceed 3 mm. Seeds or fruits smaller than 3 mm should be cracked. Avoid the use of high speed mills in the preparation of the samples to prevent the loss moisture. The portion should be large enough to be a representative sample.

AZEOTROPIC METHOD (TOLUENE DISTILLATION)

The Dean-Stark apparatus consists of a 500 mL glass flask (A) connected by a tube (D) to a cylindrical tube (B) fitted with a receiving tube (E) and a reflux condenser (C). The receiving tube (E) with 5 mL capacity is graduated in 0.1 mL divisions so that the errors of the readings do not exceed 0.05 mL. The preferred source of the heat is an electric heater with a rheostat control, or an oil-bath. The upper portion of the flask and the conical tube may be insulated.

Thoroughly clean the receiving tube and the condenser of the apparatus, rinse with water and dry. Introduce 200 mL of toluene R and about 2 mL of water into a dry flask. Heat the flask to distill liquid over a period of 2 hours. Allow to cool for about 30 min and read off the volume of water to an accuracy of 0.05 mL (first distillation).

Accurately weigh a quantity of material expected to give about 2-3 mL of water and transfer to a flask. For weighing material with a paste-like character, use a boat of metal foil. If the substance is likely to cause bumping, add enough dry, washed sand to cover the bottom of the flask or a number of capillary melting-point tubes, about 100 mm in length, sealed at the upper end. Heat the flask gently for 15 min. When boiling begins, distill at a rate of 2 drops per second until most of the water has distilled over, then increase the rate of distillation to about 4 drops per second. As soon as water has been completely distilled, rinse the inside of the condenser tube with toluene R. Continue

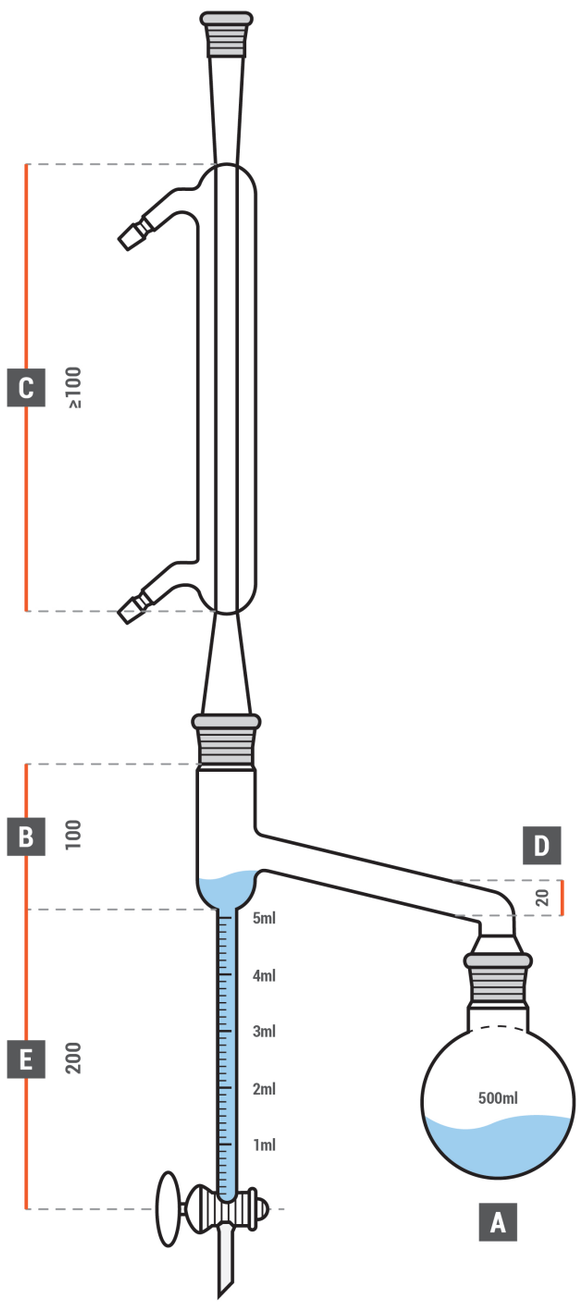

Figure 3.1. *Dean-Stark Apparatus Used to Determine Water Content by Azeotropic Method (Dimensions in mm).*
https://apps.who.int/iris/bitstream/handle/10665/44479/9789241500739_eng.pdf

the distillation for 5 more minutes, remove the heat, allow the receiving tube to cool to room temperature and dislodge any droplets of water adhering to the walls of the receiving tube by tapping the tube. Allow the water and the toluene layers to separate and read off the volume of water (second distillation). Calculate the content of water as a percentage using the formula:

$$\frac{100\,(n_1 - n)}{w}$$

where:

- w = the weight in g of the material being examined;
- n = the number of mL of water obtained in the first distillation;
- n_1 = the total number of mL obtained in both distillations.

LOSS ON DRYING FOR CRUDE PLANT DRUGS

Unless otherwise specified, transfer 2 g to 6 g of the "test sample" for analysis to a tared shallow weighing bottle, and weigh accurately. With gentle sidewise shaking distribute the "test sample" as evenly as practicable to a depth of about 5 mm generally, and not more than 10 mm in the case of bulky materials. Place the loaded bottle in the drying chamber, removing the stopper and leaving it also in the chamber. Dry the "test sample" at 105°C±2°C for 5 hours. Upon opening the chamber, close the bottle promptly, and allow it to come to room temperature in a desiccator (silica gel) before weighing. Continue the drying and weighing at 1-hour interval until the difference between two successive corresponds to not more than 0.25%. When the mass of the sample becomes constant, calculate the percentage of loss on drying using the formula:

$$\text{Loss on drying (\%)} = \frac{m_1 - m_2}{m_1} \times 100$$

where:

- m_1 = the mass of the "test sample" in g taken for analysis;
- m_2 = the mass of the "test sample" in g after drying.

<4> EXTRACTIVES

ALCOHOL-SOLUBLE EXTRACTIVES

Transfer 4 g of coarsely powdered "test sample", accurately weighed, to a glass-stoppered conical flask. Add 100 mL of 95% ethanol, and macerate for 24 hours. Insert a stopper into the flask, shaking frequently for the first 5 hours, and then allow to stand for 19 hours. Filter rapidly, taking precautions against loss of ethanol. Evaporate 25 mL of the filtrate to dryness in a tared, flat-bottomed, shallow dish and dry at 105±2°C until constant weight. Calculate the percentage of

alcohol-soluble extractive with respect to the amount of the "test sample".

$$\textit{Alcohol-soluble extractives \%} = M_e - M_c \times 100$$

where:

- M_e = the mass in grams of the extract and container after constant weight;
- M_c = the mass in grams of the container after constant weight.

WATER-SOLUBLE EXTRACTIVES

Proceed as directed under *Alcohol-Soluble Extractives*, except use water in place of ethanol.

<5> DETERMINATION OF ASH

TOTAL ASH

Accurately weigh a quantity of the "test sample", representing 24 g of the air-dried material, in a tared crucible, and incinerate, gently at first, and gradually increase the temperature to 675°C±25°C, until free from carbon, and determine the weight of the ash. If a carbon-free ash cannot be obtained this way, extract the charred mass with hot water, collect the insoluble residue on an ashless filter paper, and incinerate the residue and filter paper until the ash is white or nearly so. Then, add the filtrate, evaporate into dryness, and heat the whole to a temperature of 675°C±25°C. If a carbon-free ash cannot be obtained this way, cool the crucible, add 15 mL of alcohol, break up the ash with a glass rod, burn off the alcohol, and again heat the whole to a temperature of 675°C±25°C. Cool in a desiccator, weigh the ash, and calculate the percentage of total ash from the weight of the "test sample" taken.

ACID-INSOLUBLE ASH

Boil the ash obtained as directed in *Total Ash* with 25 mL of 3 N hydrochloric acid for 5 minutes. Collect the insoluble matter on a tared filtering crucible or ashless filter paper, wash with hot water, ignite, and weigh. Determine the percentage of acid-insoluble ash calculated from the weight of the drug taken.

WATER-SOLUBLE ASH

Boil the ash obtained as directed in *Total Ash* with 25 mL of water for 5 minutes. Collect the insoluble matter in a sintered glass crucible or on an ashless filter paper. Wash with hot water, and ignite for 15 minutes at a temperature not exceeding 450°C. Subtract the weight of this residue, in mg, obtained in *Total Ash*, and calculate the percentage of water-soluble ash with reference to the weight of sample as determined in *Total Ash*.

<6> DETERMINATION OF VOLATILE OIL

CALIBRATION PROCEDURE OF THE GRADUATED TUBE

Fill the graduated tube with distilled water up to the standard line (***Figure 3.2***) and add carefully through the sides of the tube 2 mL of xylene measured in a volumetric pipette. Open the stopcock to carefully lower the xylene to the zero (0) mark in the graduated tube. Take note of the volume of xylene in the tube. Calculate the factor using the formula:

$$f = \frac{V_t}{V_a}$$

where:

- V_t = the theoretical volume of the xylene and
- V_a = the actual volume measure in the graduated tube.

[Note: Graduated tube should be completely dry before calibration]

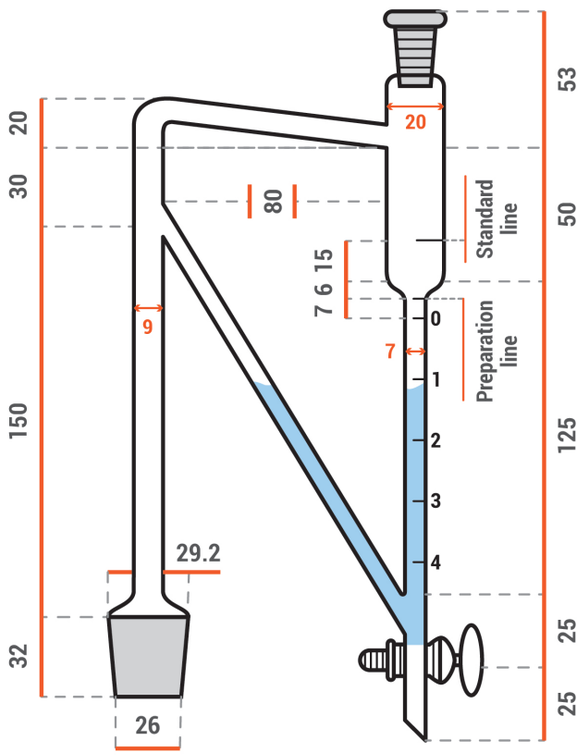

Figure 3.2. *Volatile Oil Apparatus*
(All measurements are expressed in mm)

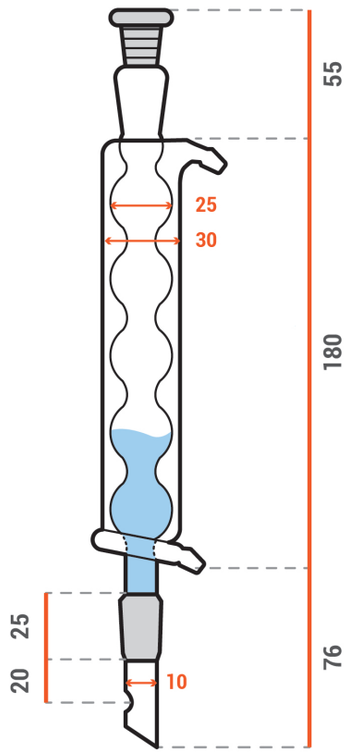

Figure 3.3. *Reflux Condenser*
(All measurements are expressed in mm)

PROCEDURE

Weigh 100 grams of the "test sample", unless otherwise specified in the individual monograph (coarsely comminute the "test sample" before weighing, if needed), place it in a 1000 mL glass-stoppered round-bottom flask, and add sufficient amount of water to submerge the sample. Small seeds, fruits, or broken leaves of herbs ordinarily need not be comminuted. When there is a need to powderize the crude drug, conduct the volatile determination within 30 minutes after pulverization. [Note: To prevent bumping, add 2 to 3 drops of silicon oil resin before analysis.]

Set up the volatile oil apparatus (***Figure 3.2***) by inserting a reflux condenser (***Figure 3.3***) in the upper mouth of the volatile oil apparatus. The graduated tube of the apparatus must be filled with water to the standard line, and add 2 mL of xylene. Heat the content of the flask in an oil bath between 130°C and 150°C for 5 hours. Remove the apparatus from the set-up. Draw off the water slowly until the surface of the oil layer corresponds to the preparation line, and allow it to stand for more that 1 hour at room temperature. Then, lower the surface of the oil layer to the zero line, and read the volume (mL) of the oil. Subtract the volume (mL) of xylene from the volume of the total oil.

$$\textit{Volatile Oil Content} = \textit{(total volume xf)} - \textit{2 mL (volume of xylene)}$$

<7> DETERMINATION OF SWELLING CAPACITY

Swelling index is the volume in milliliters (mL) taken up by the swelling of 1 g of herbal material in water or in other specified solvent at a definite time and temperature.

Perform simultaneously no less than three determinations for any given material. Introduce the specified quantity of the herbal material, reduced to the required fineness, accurately weighed, into a 25 mL glass-stoppered measuring cylinder. The internal diameter of the cylinder should be about 16 mm, length of the graduated portion is about 125 mm, marked in a 0.2 mL divisions from 0 to 25 mL in an upward direction. Unless otherwise indicated in the test procedure, add 25 mL of water or specified solvent at 20 -25°C, stopper tightly, shake and stand. Shake the mixture thoroughly every 10 min for 1 hour. Allow to stand for 3 hours at room temperature, or as specified. Measure the volume in mL occupied by the herbal material, including any sticking mucilage. Calculate the mean value of the individual determinations, related to 1 g of herbal material.

$$S = \frac{V}{W}$$

Where:

- S = the swelling capacity;
- V = the swelling volume of the test specimen (mL);
- W = the weight of the test specimen calculated in dry basis (g).

<8> DETERMINATION OF BITTERNESS INDEX

The bitterness properties of herbal materials are determined by comparing the threshold of the bitter concentration of extract of the materials with that of a dilute solution of quinine hydrochloride R. The bitterness value is expressed in units equivalent to the bitterness of a solution containing 1 g of quinine hydrochloride R in 2000 mL.

The sensitivity to bitterness can vary between individuals or even for an individual in different situations (fatigue, smoke, food intake). Therefore, the determination of the threshold concentration of the bitterness of the material to be tested with quinine hydrochloride should be made by the same person within a short space of time. A certain amount of training is required to perform the test. An individual who dislikes a bitter sensation induced by a solution containing 0.058 mg of quinine hydrochloride in 10 mL of water is not well suited to take part in the determination.

The preparation of the stock solution of each individual herbal material (S_I) should be specified in the test procedure. In each test series, unless otherwise indicated, the determination should start with a lower concentration in order to retain sufficient sensitivity in the taste buds.

Caution: The test should not be carried out until the identity of the herbal material has been confirmed.

PREPARATION OF SOLUTIONS

► ***Stock and Diluted Quinine Hydrochloride Solutions***

Dissolve 0.1 g of quinine hydrochloride R in sufficient quantity of safe drinking water to produce 100 mL. Further dilute 5 mL of this solution to 500 mL of safe drinking water. This standard solution of quinine hydrochloride (S_q) contains 0.01 mg/mL. Use nine test tubes for the serial dilution for initial test as indicated in ***Table 3.3.***

Table 3.3. Determination of Bitterness Value: Serial Dilution for the Initial Test

	TUBE NO.								
	1	2	3	4	5	6	7	8	9
S_q (mL)	4.2	4.4	4.6	4.8	5.0	5.2	5.4	5.6	5.8
Safe drinking water (mL)	5.8	5.6	5.4	5.2	5.0	4.8	4.6	4.4	4.2
Quinine hydrochloride in 10 mL of solution (=*c*) (mg)	0.042	0.044	0.046	0.048	0.050	0.052	0.054	0.056	0.058

S_q = stock solution of quinine hydrochloride.

► ***Stock Solution and Diluted Solution of Plant Material***

Prepare a stock solution as specified in the test procedure for the given plant material (ST). Use 10 test tubes for the serial dilution for the test as shown in ***Table 3.4*** below.

Table 3.4. *Determination of Bitterness Value: Serial Dilution for the Second Test*

	Tube no.									
	1	2	3	4	5	6	7	8	9	10
S_q (mL)	1.00	2.00	3.00	4.00	5.00	6.00	7.00	8.00	9.00	10.00
Safe drinking water (mL)	9.00	8.00	7.00	6.00	5.00	4.00	3.00	2.00	1.00	–

S_q = stock solution of the herbal material examined.

PROCEDURE

After rinsing the mouth with safe drinking water, taste 10 mL of the most dilute solution, swirling it in the mouth mainly near the base of the tongue for 30 seconds. If the bitter sensation is no longer felt in the mouth after 30 seconds, spit out the solution and wait for 1 min to confirm whether this is due to delayed sensitivity. Rinse with safe drinking water. The next highest concentration should not be tasted until at least 10 minutes has passed. The threshold bitter concentration is the lowest concentration at which the material continues to produce a bitter sensation after 30 seconds. After the first series of tests, thoroughly rinse the mouth with safe drinking water until no bitter sensation remains. Wait for at least 10 min before performing the second test.

For time efficiency, it is recommended to demonstrate whether the solution contained in test tube no. 5 (containing 5 mL of ST in 10 mL) gives a bitter sensation. If such, determine the threshold bitter concentration of the material by tasting the dilutions in tube numbers 1-4. If the solution in test tube no. 5 does not give a bitter sensation, locate the threshold bitter concentration by tasting the dilutions in test tube numbers 6-10.

All solutions including the drinking water for mouth washing should be at 20-25°C.

Calculate the bitterness value in units per g using the following formula:

$$\frac{2000 \times c}{a \times b}$$

where:

- a = the concentration of the stock solution (S_T) (mg / mL);
- b = the volume of S_T (in mL) in the tube with the threshold bitter concentration;
- c = the quantity of quinine hydrochloride R (in mg) in the tube with the threshold bitter concentration.

<9> DETERMINATION OF TANNINS

Tannins are soluble, astringent complex phenolic substances of plant origin used in tanning of animal skins or precipitation of proteins. These are complex, easily oxidized and polymerized in solution. if these happen, they lose much of their astringent effect and are therefore of little therapeutic value.

PROCEDURE

Prepare the material extract by introducing a specified quantity of the herbal material concerned, previously powdered to a known fineness into a conical flask. Add 150 mL of water and heat over a boiling water-bath for 30 min. Cool, transfer the mixture to a 250 mL volumetric flask and dilute to a volume with water. Allow the solid material to settle and filter the liquid through a filter paper with 12 cm diameter. Discard the first 50 mL of filtrate.

To determine the total amount of material that is extractable in water, evaporate 50 mL of the plant material extract to dryness. Dry the residue in an oven at 105°C and weigh (T_1).

To determine the amount of herbal material not bound to hide powder* that is extractable in water, take 80 mL of the herbal material extract. Add 6 g of hide powder R and shake well for 60 min. Filter and evaporate 50 mL of the clear filtrate to dryness. Dry the residue in an oven at 105°C and weigh (T_2).

To determine the solubility of the hide powder, take 6 g of the hide powder R, add 80 mL of water and shake well for 60 min. Filter and evaporate at 50 mL of the clear filtrate to dryness. Dry the residue in an oven at 105°C and weigh (T_0).

Calculate the quantity of tannins as a percentage using the following formula:

$$\frac{[T_1 - (T_2 - T_0)] \times 500}{W}$$

where:

- W = the weight of herbal material in grams.

* Hide Powder R.
A suitable commercially available reference material.

II. Methods in Pharmacognosy

In compliance with the methods in pharmacognosy, the raw materials and products obtained from the raw materials shall undergo macroscopic and microscopic examinations, pharmacognostic and physicochemical studies and component analyses using spectroscopic and chromatographic techniques.

<21> GUIDELINES FOR COLLECTION OF PLANT MATERIALS

The selected species for collection should be identical to the ones specified in the national pharmacopeia. Sustainable, non-destructive systems of collection should be applied. Plant materials for collection should be healthy and well-developed.

Medicinal plants found in areas where high levels of pesticides or other possible contaminants are used or found, such as roadsides, catchwater drains, landfills, and industrial facilities, should not be collected. Further, those found in and around active pastures, including riverfronts downstream from pastures, should be avoided as these may be contaminated with animal excreta.

Discard plant parts that are not required, show signs of insect infestation or fungal growth, and foreign matter such as toxic weeds. Decomposed medicinal plant materials should be removed.

<22> SAMPLING FROM THE COLLECTED PLANT MATERIALS

To ensure the best quality of medicinal plant materials, collection of plant materials for samples should be done in its optimal season since the time of harvest depends on the plant part to be used. The quality peak season and time of day should be determined according to the quality and quantity of the active ingredient and not the total yield of the materials gathered. The concentration of biologically active components differs with the stage of plant growth and development. The same is true for non-targeted toxic or poisonous indigenous plant ingredients. Care should be taken to ensure that the collected medicinal plants for sampling are not admixed with foreign matter, weeds or toxic plants.

Also, harvest the medicinal plants for sampling under best possible climatic conditions to avoid microbial fermentation and molds which could lead to degradation of the material.

The main roots of trees and bushes should not be cut or dug up when collecting samples. Dig the root at a substantial distance, approximately 30 cm from the main stem or taproot to avoid breakage. Only some of the lateral roots should be located and collected. Cover up the hole after digging to protect against infection and invasion of pests.

When collecting species whose bark is the primary material to be used, avoid ring barking or girdling, where the entire rings of the bark are removed from the tree. Peel the bark from the tree in small longitudinal strips using a thin flexible blade or bush knife. Remove the bark in small sections and leave some inner bark to protect the wood.
For leaf collection, pluck individual leaves instead of leaf striping and avoid the use of sharp pruning shears. Prune branches regularly to improve quality and quantity of the leaves.

Storage conditions are very important for microbiological quality, to preserve secondary metabolites and conserve

medicinal plants, keep them dry as far as possible, as fresh samples are fragile and is prone to accelerated degradation. Poor storage and drying can lead to spoilage, biological contamination, photo-decomposition or atmospheric oxidation.

<23> SAMPLING OF PLANT MATERIALS IN BULK CONTAINERS FOR TESTING

The reliability of any conclusions drawn from the analysis of a sample will depend upon how well the sample represents the whole batch. Special handling procedures are required in relation to sampling because of the specific characteristics of medicinal plant materials, in particular their lack of homogeneity. The following procedures should be observed when selecting and preparing an average sample from a batch of plant material.

Inspect each container or packaging unit for conformity with pharmacopeia monographs or other requirements regarding packaging and labeling. Check the condition of the package and note any defects that may influence the quality or stability of the contents (physical damage, moisture, etc.). Sample damaged containers individually.

If initial inspection indicates that the batch is uniform, take samples as follows. When a batch consists of five containers or packaging units, take a sample from each one. From a batch of 6-50 units, take a sample from five. In the case of batches of over 50 units, sample 10%, rounding up the number of units to the nearest multiple of ten. For example, a batch of 51 units would be sampled as for 60, i.e. take samples from six packages.

After opening, inspect the contents of the units selected for sampling for:

- organoleptic characteristics (color, texture and odor);
- presentation of the material (raw, cut, crushed, compressed);
- the presence of admixtures, foreign matter (sand, glass particles, dirt), mould, or signs of decay;
- the presence of insects;
- the packaging material originating from poor or degraded containers.

From each container or package selected, take three original samples, taking care to avoid fragmentation. Samples should be taken from the top, middle and bottom of the container. In the case of sacks and packages, the three samples should be taken by hand, the first from a depth of not less than 10 cm from the top and the second and third from the middle and bottom after cutting into the side of the package. Samples of seeds should be withdrawn with a grain probe. Material in boxes should first be sampled from the upper layer; then approximately half of the contents should be removed and a second sample taken. Finally after further removal of material, another sample should be taken from the bottom. Samples should be as uniform as possible in mass. The three original samples should then be combined into a pooled sample which should be mixed carefully.

The average sample is obtained by quartering. Form the pooled sample, adequately mixed, into an even and square-shaped heap, and divide it diagonally into four equal parts. Take two diagonally opposite parts and mix carefully. Repeat the process as necessary until the required quantity, to within ±10%, is obtained (100-200 g for flowers and up to 10 kg for certain roots). Any remaining material should be returned to the batch.
Using the same quartering procedure, divide the average sample into four final samples, taking care that each portion is representative of the bulk material. The final samples are tested for the following characteristics:

- degree of fragmentation (sieve test);
- identity and level of impurities;
- moisture and ash content;
- level of active ingredients, where possible.

A portion of each final sample should be retained to serve as reference material, which may also be used for re-testing purposes if necessary.

<24> SAMPLING OF MATERIALS IN RETAIL PACKAGES FOR TESTING

From each wholesale container (boxes, cartons, etc.) selected for sampling, take at random two consumer packages at random. From small batches (1-5 boxes), take 10 consumer packages. Prepare the pooled sample by mixing the contents of the selected consumer packages and proceed as described above to obtain the final sample.

<25> PROCESSING/PACKAGING/ LABELING/STORAGE/DISTRIBUTION

PROCESSING

1. Processing includes washing, cutting before drying, fumigation, freezing, distillation, drying, etc. Where applicable, all of these processes must conform to government regulations and should be carried out soon after harvesting the medicinal plants.

2. Upon arrival at the processing facility, the harvested medicinal plant/herbal substance has to be promptly unloaded and unpacked. Prior to processing, the material should not be exposed directly to the sun, except in cases where there is a specific need, and must be protected from rainfall, insect infestation, etc.

3. For natural open-air drying, the medicinal plant/herbal substance must be spread out in a thin layer. In order to secure adequate air circulation, the drying frames must be located at a sufficient distance from the ground. Drying directly on the ground or under direct exposure to the sunlight should be avoided unless specifically required. Attempts must be made to achieve uniform drying of the medicinal plant/herbal substance and thus, avoid mold formation.

4. Except in the case of open-air drying, the drying conditions such as temperature, duration, air circulation, etc., must be selected, taking into consideration the medicinal plant parts such as root, leaf, or flower and the nature of its active constituents such as essential oils. Individual conditions must be recorded in detail.

5. All materials must be inspected and, where necessary, sieved in order to eliminate substandard product and foreign bodies. Sieves must be maintained in a clean state and should be serviced regularly.

6. Clearly marked waste bins should be available, emptied daily, and cleaned.

PACKAGING / LABELING

The specific geographic location of the collection area of medicinal plant/herbal substance and the harvest period should be indicated as precisely as possible. For batches of medicinal plant materials, they should be labeled and traceable to their sources. Appropriate labeling and batch assignment should be indicated properly and be done as early as possible. It is advisable that the collected wild medicinal plant and cultivated medicinal plant material should carry different batch numbers.

1. For protection of the product and to reduce the risk of pest attacks, early packaging is advisable.

2. Following processing monitored by in-process controls, the product should be in clean and dry packages, preferably new sacks, bags or cases. The label must be clear, permanently fixed and made from non-toxic material. Information must conform with regional and/or national labeling regulations.

3. Reusable packaging material should be well cleaned and properly dried prior to use. No contamination should occur through reusing of bags.

4. Packaging materials must be stored in a clean and dry place that is free of pests and inaccessible to livestock and domestic animals. It must be guaranteed that no contamination of the product can occur because of the use of packaging materials, particularly in the case of fiber bags.

5. Labels affixed shall include but not limited to: scientific name of the medicinal plants/herbal substance, net weight, recommended storage condition, batch number, expiry date, etc.

STORAGE AND DISTRIBUTION

1. Storage area should be of sufficient capacity to allow orderly storage. It must be well-lit and within the humidity limit.

2. Medicinal plants/herbal substances should be controlled, monitored and recorded, where appropriate, to ensure good storage conditions, and to comply with the "first-in" and "first-out" principle.

3. Packaged dried medicinal plants/herbal substances, including essential oils, should be stored in a dry, well-aerated building, in which daily temperature fluctuations are limited and good aeration is ensured.

4. Fresh plant materials should be stored between 2°C-8°C while frozen products should be stored below -20°C for long-term storage.

5. It is important to secure dry conditions for bulk transport. To reduce the risk of mold formation or fermentation, it is advisable to use aerated containers. As a substitute, the use of sufficiently aerated transport vehicles and other aerated facilities is recommended. Essential oil transport must conform with appropriate regulations. Regional and/or national regulations on transport have to be observed.

6. Segregated storage should be exercised for different kinds of material to avoid cross-contamination.

7. Storage areas should be protected against any insects, birds, rodents, and domestic animals. Regular on-spot monitoring is required to check any pest infestation, fungal attack, or rotting.

8. Fumigation against pest attack should be carried out only where necessary and must be carried out exclusively by licensed personnel using registered chemicals. Any fumigation against pest attack should be reported in the documentation.

9. When frozen storage or saturated steam is used for pest control, the humidity of the material must be controlled after treatment.

Adapted from the following references:

WHO Guidelines on Good Agricultural and Collection Practices (GACP) for Medicinal Plants WHO, Geneva (2003)
WHO Guidelines on Quality Control Methods for Medicinal Plants WHO, Geneva (2003)
Philippine Pharmacopeia 1 with Supplement (2005)
USP Herbal Medicine Compendium https://hmc.usp.org/about/general-chapters
The Japanese Pharmacopeia 17th edition English version

<26> MACROSCOPIC EXAMINATION

The macroscopic study involves the morphological description of the plant parts carried out by the naked eye preferably placed on a white paper surface with proper illumination. The organoleptic features such as the size, shape, color, measurements, texture, odor of plant parts (e.g., branches, leaves, flowers, fruits and seeds) should be examined.

GENERAL FEATURES

A vital means of plant identification is to include both vegetative (roots, stem, leaves) and reproductive (flowers, fruits, seeds) morphology. This will ensure determination with certainty whether the range of variation of the unknown plant corresponds to that listed in the description of a known plant (Simpson, 2019). Descriptive works include diagnosis, vegetative and reproductive descriptions, voucher specimen, locality data, and figures of structures essential in characterizing a plant species.

DIAGNOSTIC FEATURES

Reading the entire written descriptions of a plant can be time consuming. A good practice relies on narrowing down the possibilities first by identifying its diagnostic characteristics from a long list of features. Diagnosis involves considering the outstanding features of the plant that make them unique from all other entities. A plant species can be recognized by some unique combination of both vegetative and reproductive characters.

VISUAL EXAMINATION AND MICROSCOPIC INSPECTION

The identity, purity and quality of a plant material must be established through detailed visual examination, both macroscopic and microscopic. Whenever possible, the plant material must be compared with the authentic raw material which comes from a sample identified in the Pharmacopeia. Any plant that shows discrepancy in color, consistency, odor and flavor should be discarded.

The macroscopic identification of drugs when whole, is based on shape, size, color, texture, surface characteristics, fracture characteristics, and appearance of cut surface. As these characteristics of identification are subjective and substitutes or adulterants may closely resemble the genuine material, it is necessary to simultaneously carry out microscopic physico-chemical analysis of the sample.

The microscopic inspection is indispensable for the identification of broken or powdered material and the specimen may have to be treated with chemical reagents.

(A) SIZE

Measurements of length, width and thickness using a graduated ruler in millimeters is sufficient for measuring. Small seeds and fruits may be measured by aligning 10 of them on a sheet of calibrated paper with 1 mm spacing between the lines and dividing the results by 10.

(B) COLOR

Examine the raw material before any treatment, under diffused daylight or under artificial light source with wavelengths similar to the light of day. The color of the sample should be compared with the reference material.

(C) SURFACE CHARACTERISTICS

Examine the raw material before any treatment. When necessary, use a lens with six up to 10 times magnification. When indicated in the monograph, moisten with water or reagent specified to observe characteristics of the surface of the fracture. Touch the material to check whether it is soft or hard, bend and break it to acquire information about the brittleness and appearance of the fracture plane, whether it is fibrous, smooth, rough, and granular, among others.

(D) ODOR

Before checking the odor of the material, make sure that there is no risk. Place a small amount of sample in the palm of your hand or in a glass container and waft the air over the material with your hand slowly and repeatedly. If odor is indistinctive, crush the material between the thumb and index fingers or between the palms of the hands using gentle pressure and inhale again. If the monograph indicates a toxic material, crushed by mechanical means and placed in a beaker then pour a small quantity of boiling water onto the crushed material. First, determine the strength of the odor: none, weak, distinct or strong and then the sensation caused by the odor: aromatic, fruity, musty. Moldy, rancid or stale. When possible, it is important to compare the odor with a defined substance, such as, for example, peppermint must have an odor similar to menthol and clove, similar to eugenol.

(E) TASTE

Only applied to herbal material specifically required in the monograph.

<27> MICROSCOPIC EXAMINATION

The microscopic study pertains to the anatomical examination by making sections of the plant parts to be studied. A transverse section of plant parts such as leaves and flowers can be treated with a chloral hydrate solution for at least an hour, and subsequently placed on microscopic slides and properly stained. Microscopic preparations of branches and fruits can be done using a microtome. Prepared slides covered by coverslip should be examined using either a dissecting or compound microscope. Slides examined can be simultaneously photographed by a digital camera with a good resolution. Anatomical examination of smaller plant parts such as stamen, pistil, seeds should be done using a compound microscope while anatomical architecture of pollens can be examined best using a scanning electron microscope.

DESCRIPTION

Understanding the general anatomy of plant parts is essential in recognizing the distinguishing structures of identifying and evaluating individual crude plant drugs in histological sections and also in powdered form.

ILLUSTRATION

Microscopic photographs or diagrammatic illustrations of plant parts such as the transverse section of the stem, bark, leaf, flowers, fruits and seeds are useful for identification. The diagnostic structures of powdered crude drugs may include epidermal cells, crystal cells, parenchyma cells, fiber or stone cells, among others.

(A) PREPARATION OF THE MATERIAL FOR MICROSCOPIC ANALYSIS

SOFTENING OF THE MATERIAL

Typically, the organs and tissues of the plant used for microscopic examination are dried. Soften by treating them with hot water. The time required to soften each plant organ or any of its parts varies, depending on its texture.

METHOD OF HYDRATION FOR DRY MATERIALS

For hard material such as bark, wood, and others, place the sample in a solution of hydration, prepared with equal parts of water, ethanol, and glycerin and five drops of commercial detergent for every 200 mL of solution. Soak for a few hours or overnight until they become soft enough for cutting. Boiling in water for a few minutes may sometimes be necessary.

IMPLEMENTATION OF CUTS

Once soft, proceed to preparation of slices of the plant organs or parts thereof. Select representative pieces of the material to be examined and cut into appropriate lengths, with one softened and smoothed. Free hand cutting can be made with the aid of a razor blade or scalpel. Sections of better quality can be obtained with the employment of microtomes. Prepare a cross or transverse section by cutting at a right angle to the longitudinal axis of the material. Longitudinal sections can be prepared by cutting in parallel to the longitudinal axis, either in a radial direction (radial section) or in a tangential direction (tangential section).

Wood, woody stems, rhizomes and roots can be cut by holding the material between the thumb and index finger, supported by the middle finger or by holding the central hole of a hand microtome. Thin materials such as leaves, petals and slender stems should be bound between two halves of a piece of elder-pith or other suitable support. If necessary, moisten the surface to be cut and the blade with ethanol (~375 g/L) TS. Cut the sections as thinly and evenly as possible. Transfer the sections with a brush moistened with ethanol (~150 g/L) TS to a dish containing ethanol (~150 g/L) TS. Select suitable sections for the preparation of the slides. For certain materials, a sliding microtome may be used.

Seeds and fruits that are very flat, or that are small and spherical, and cannot be held in the manner described above may be inserted into a notch cut into a small rubber stopper or embedded in hard paraffin (paraffin wax).

Prepare a hard paraffin block, rectangular in shape, measuring about 7×7×15 mm, and melt a small hole in the center of one end using a heated needle or thin glass rod. Press the material, which should be dry or softened by exposure to moisture, into this hole. Then, prepare sections with a microtome.

For the examination of mucilage, aleurone grains or spherical aggregations of inulin, cut the material without using water.

(B) CHARACTERIZATION OF MICROSCOPIC PARTICLES

The presence of certain cell contents, such as starch grains, aleurone grains, plastids, fats and oils, may render sections non-translucent and conceal certain characteristics. To make the remaining parts stand out clearly or produce a penetrating effect, use reagents that dissolve some of these contents. This turns the section more transparent and reveals details of the structures.

If the refractive index of the clarifying agent is close to that of the cell structure, the material being tested becomes almost invisible; if it differs significantly, the material becomes markedly evident.
The most frequently used clarifying agents are described below. (For the preparation of reagents, refer to *Reagents and solutions* under *General Information/Appendix*).

CHLORAL HYDRATE TS

On gentle heating, chloral hydrate TS dissolves starch grains, aleurone grains, plastids, and volatile oils, and expands collapsed and delicate tissue without causing any undue swelling of cell walls or distortion of the tissues. It has a refractive index (n_D) of 1.44–1.48. It is the best reagent for rendering calcium oxalate clearly evident and is particularly useful for small crystals. However, when allowed to stand, it slowly dissolves calcium oxalate, owing to an increase in acidity.

LACTOCHLORAL TS

Lactochloral TS has a similar use with chloral hydrate TS, but is usually applied to sections that are difficult to clarify. It may be used cold. Before use, any air present in the specimen should be removed by placing in a desiccator and applying a vacuum.

LACTOPHENOL TS

Lactophenol TS may be used cold or with heating. It has a refractive index (n_D) of 1.44 and is useful for the preparation of fungi, pollen grains, most non-oily powders, and parasites such as mites and nematode worms. Sizes of starch grains can be measured accurately, but the concentric rings are usually invisible when prepared in this reagent. Crystals of calcium oxalate are clearly visible in lactophenol and shine brightly when illuminated with polarized light. This reagent dissolves calcium carbonate deposits with a slow effervescence, owing to the presence of lactic acid.

SODIUM HYPOCHLORITE TS

Sodium hypochlorite TS is used for bleaching deeply colored sections. Immerse the sections in the solution for a few minutes until sufficiently bleached, wash with water and prepare the mount with glycerol/ethanol TS. The bleached sections give a negative reaction to lignin.

SOLVENTS FOR FATS AND OILS

Xylene R and light petroleum R can be used to remove fats and oils from oily powders or sections. When necessary, immerse the material in the solvent for a short time, decant the liquid and wash the material with fresh solvent.

(C) HISTOCHEMICAL DETECTION OF CELL WALLS AND CONTENTS

Reagents can be applied to a powdered sample or a section on a slide using the following methods:

- Add drops of the reagent to the sample and apply a cover glass. Irrigate using a strip of filter paper as described below.
- Place drops of the reagent on one edge of the cover glass of a prepared specimen. Place a strip of filter paper at the opposite edge of the cover glass to remove the fluid under the cover glass by suction, causing the reagent to flow over the specimen.
- Using the second method, the progress of the reaction may be observed under a microscope. Care should be taken to avoid using reagents or vapors that could attack the lenses or stages of the microscope.

CELLULOSE CELL WALLS

Add 1–2 drops of iodinated zinc chloride TS and allow to stand for a few minutes. Alternatively, add 1 drop of iodine (0.1 mol/l) VS, allow to stand for 1 min, remove excess reagent with a strip of filter paper and add 1 drop of sulfuric acid (~1160 g/l) TS; cellulose cell walls are stained blue to blue-violet. On the addition of 1–2 drops of cuoxam TS, the cellulose cell walls will swell and gradually dissolve.

LIGNIFIED CELL WALLS

Moisten the powder or section on a slide with a small volume of phloroglucinol TS and allow to stand for about 2 min or until almost dry. Add 1 drop of hydrochloric acid (~420 g/l) TS and apply a cover glass; lignified cell walls are stained pink to cherry red.

SUBERIZED OR CUTICULAR CELL WALLS

Add 1–2 drops of sudan red TS and allow it to stand for a few minutes or warm gently; suberized or cuticular cell walls are stained orange-red or red.

ALEURONE GRAINS

Add a few drops of iodine/ethanol TS. The aleurone grains will turn yellowish brown to brown. Then, add a few drops of ethanolic trinitrophenol TS; the grains will turn yellow. Add about 1 mL of mercuric nitrate TS and allow to dissolve; the color of the solution turns brick red. If the specimen is oily, render it fat-free by immersing and washing it in an appropriate solvent before carrying out the test.

CALCIUM *CARBONATE*

Crystals or deposits of calcium carbonate dissolve slowly with effervescence when acetic acid (~60 g/l) TS or hydrochloric acid (~70 g/l) TS is added.

CALCIUM *OXALATE*

Crystals of calcium oxalate are insoluble in acetic acid (~ 60g/l) TS but dissolve in hydrochloric acid (~70 g/l) TS without effervescence (if applied by irrigation the acid should be more concentrated). They also dissolve in sulfuric acid (~350 g/l) TS, but needle-shaped crystals of calcium sulfate separate on standing after about 10 min. In polarized light, calcium oxalate crystals are birefringent. Calcium oxalate is best viewed after the sample has been clarified (e.g. with chloral hydrate TS).

FATS, FATTY OILS, VOLATILE OILS AND RESINS

Add 1–2 drops of sudan red TS and allow to stand for a few minutes or heat gently, if necessary. The fatty substances are stained orange-red to red. Irrigate the preparation with ethanol (~750 g/l) TS and heat gently. The volatile oils and resins dissolve in the solvent, while fats and fatty oils (except castor oil and croton oil) remain intact.

HYDROXYANTHRAQUINONES

Add 1 drop of potassium hydroxide (~55 g/l) TS; cells containing 1,8-di- hydroxyanthraquinones are stained red.

INULIN

Add 1 drop each of 1-naphthol TS and sulfuric acid (~1760 g/l) TS; spherical aggregations of crystals of inulin turn brownish red and dissolve.

MUCILAGE

Add 1 drop of Chinese ink TS to the dry sample. The mucilage shows up as transparent, spherically dilated fragments on a black background. Alternatively, add 1 drop of thionine TS to the dry sample, allow to stand for about 15 min, and then wash with ethanol (~188 g/l) TS. The mucilage turns violet-red (cellulose and lignified cell walls are stained blue and bluish violet respectively).

STARCH

Add a small volume of iodine (0.02 mol/l) VS; a blue or reddish blue color is produced. Alternatively, add a small volume

of glycerol/ethanol TS and examine under a microscope with polarized light; birefringence is observed giving a Maltese cross effect with the arms of the cross intersecting at the hilum.

TANNIN

Add 1 drop of ferric chloride (50 g/l) TS; it turns bluish black or greenish black.

(D) DISINTEGRATION OF TISSUES

Cut the material into small pieces about 2 mm thick and 5 mm long, or into slices about 1 mm thick (tangential longitudinal sections are preferred for woods or xylem from stems).

Use one of the following methods depending on the nature of the cell walls. For tissues with lignified cell walls, use either method 1 or method 2. For tissues where lignified cells are few or occur in small groups, use method 3.

METHOD 1. NITRIC ACID AND POTASSIUM CHLORATE

Place the material in a test tube containing about 5 mL of nitric acid (~500 g/L) TS and heat to boiling. Add a small quantity of powdered potassium chlorate R and allow to react, warming gently if necessary to maintain a slight effervescence. Add fresh quantities of powdered potassium chlorate R as needed. When the tissue appears to be almost completely bleached and shows a tendency to disintegrate, apply pressure to the material with a glass rod. If the material breaks, interrupt the reaction by pouring the contents of the test tube into water. Allow the material to settle, decant it and wash it with fresh water until the acidity is removed. Transfer the material onto a slide and tease it out with a needle. Add 1 drop of glycerol/ ethanol TS and apply a cover glass. The disintegrated material gives a negative reaction for lignin.

METHOD 2. NITRIC ACID AND CHROMIC ACID

Place the material in a small dish and heat with nitro-chromic acid TS until the material breaks easily when pressure is applied with a glass rod. Wash the material repeatedly with water and transfer onto a slide. Tease out the material, add 1 drop of glycerol/ethanol TS and apply a cover glass. The disintegrated material gives a negative reaction for lignin.

This treatment can also be carried out on a slide. Place a moderately thick section of the material on a slide, add the reagent and allow it to stand for about 20 min. Separate the cells by applying gentle pressure, or with a sliding movement of the cover glass. This process is especially useful when the disintegration of the tissues of a section under the microscope needs to be observed to ascertain where isolated cells come from.

METHOD 3. CAUSTIC ALKALI METHOD

Place the material in a test tube containing about 5 mL of potassium hydroxide (~110 g/l) TS or sodium hydroxide (~80 g/l) TS, and heat on a water-bath for 15–30 min until a portion breaks easily when pressure is applied with a glass rod. Decant the liquid and wash the softened material several times with fresh quantities of water. This method is particularly useful for the disintegration of bark, seeds, leaves and flowers, facilitating the elimination of fibers, scleroids, lactiferous tissues and epidermis. The disintegrated material gives a negative reaction for lignin.

(E) MEASUREMENT OF SPECIMENS

EQUIPMENT

Use a microscope with an ocular micrometer to measure the size of small objects. The scales should be calibrated using a stage micrometer, consisting of a glass slide of usual size, upon which a scale is engraved or photographed, usually 1 or 2 mm long, in 0.1 and 0.01 mm divisions. The ocular micrometer consists of a small disc of glass, across the diameter

of which a 100-line scale is engraved or photographed. The disc is placed into the eyepiece.

CALIBRATION OF THE OCULAR MICROMETER

Place the ocular micrometer scale in the eyepiece of the microscope. Put the stage micrometer on the microscope stage and focus on the scale divisions. Both scales should be sharply defined in the field of view. Turn the eyepiece to place the scales in a parallel position and, if necessary, move the stage micrometer until the starting lines of both scales coincide (***Figure 3.4***). Now find another point, as far along the scale as possible, where two other division lines are exactly superimposed. Count the number of divisions on the ocular micrometer and the corresponding length on the stage micrometer scale, in order to determine the length that is equivalent to one division on the ocular micrometer scale. For example, if 100 divisions on the ocular micrometer scale are equal to 30 divisions on the stage micrometer scale, since the divisions on the stage micrometer scale are 0.01 mm apart, 100 ocular micrometer divisions are equivalent to 0.30 mm and therefore each division on the ocular micrometer scale represents 3.0 µm. Since the calibrations apply only for a particular lens combination, it is advisable to determine and record the ocular micrometer values for the most frequently used combinations.

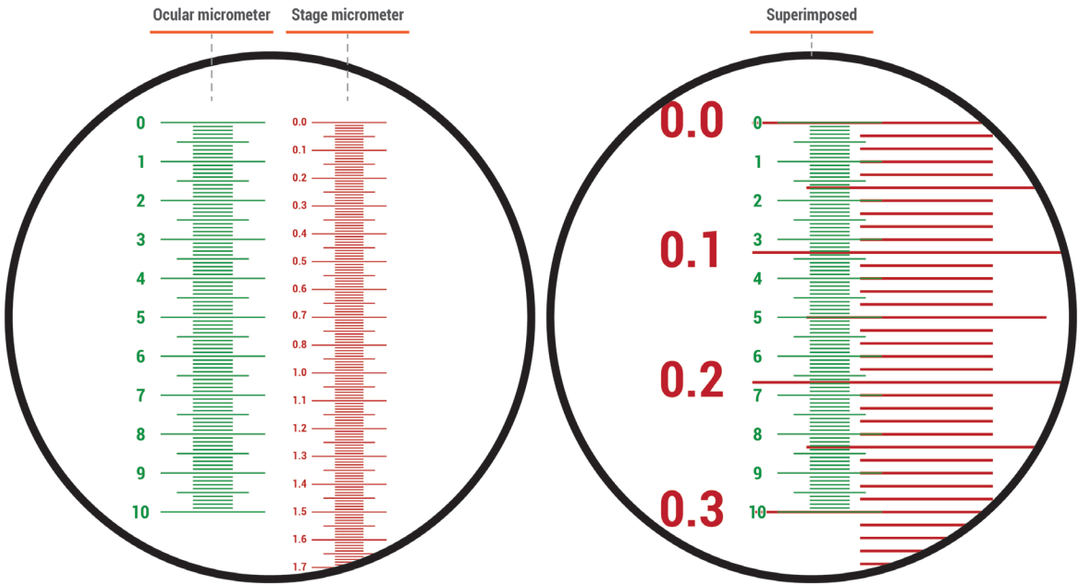

Figure 3.4. *Alignment of the Stage Micrometer and the Ocular Micrometer*

METHOD

Place the specimen on the microscope stage and focus on the object to be measured. Superimpose the ocular micrometer scale and read off the dimensions of the object. Multiply the number of scale divisions by the micrometer value to give the actual dimension in micrometers. By this method, using a 40× objective and a 6× eyepiece, measurements are correct to the nearest 2 µm, i.e. a dimension of 20 µm is liable to an error of 2 µm, or 10%, or ±5%; a dimension of 100 µm is liable to an error of 2% or ±1%.

For curved and elongated objects, the measurement of lengths may be made using a microscope equipped with a drawing apparatus or a camera lucida. The instrument should be set up so that the image of the object, the drawing paper and the pencil are in focus simultaneously. With the stage micrometer in place, trace the lines of the stage micrometer scale upon the paper fastened on a drawing board. Tilt the drawing board, if necessary, until the divisions drawn upon the paper are equally spaced. The magnification is determined by measuring the distance between selected lines on the drawing paper

and dividing by the distance between the corresponding lines on the stage micrometer. Place the specimen on the microscope stage and trace the image of the object on the paper. Superimpose a colored thread along the length of the object drawn on the paper and, after straightening it, measure the length of the thread by means of a ruler graduated in millimeters. Divide the measured length by the magnification to determine the actual length of the object.

LEAF STOMATA

► Types of Stoma

In the mature leaf, four significantly different types of stoma are distinguished by their form and the arrangement of the surrounding cells, especially the subsidiary cells (***Figure 3.5***).

a. The *anomocytic* or *ranunculaceous (irregular-celled) type* - the stoma is surrounded by a varying number of cells, generally not different from those of the epidermis.

b. The *anisocytic* or *cruciferous (unequal-celled) type* - the stoma is usually surrounded by three or four subsidiary cells, one of which is markedly smaller than the others.

c. The *diacytic* or *caryophyllaceous (cross-celled) type* - the stoma is accompanied by two subsidiary cells, the common wall of which is at right angle to the stoma.

d. The *paracytic* or *rubiaceous (parallel-celled) type* - the stoma has two subsidiary cells, of which the long axes are parallel to the axis of the stoma.

In describing an epidermis where certain stomata differ from the predominant type, the term applying to the majority of stomata is used.

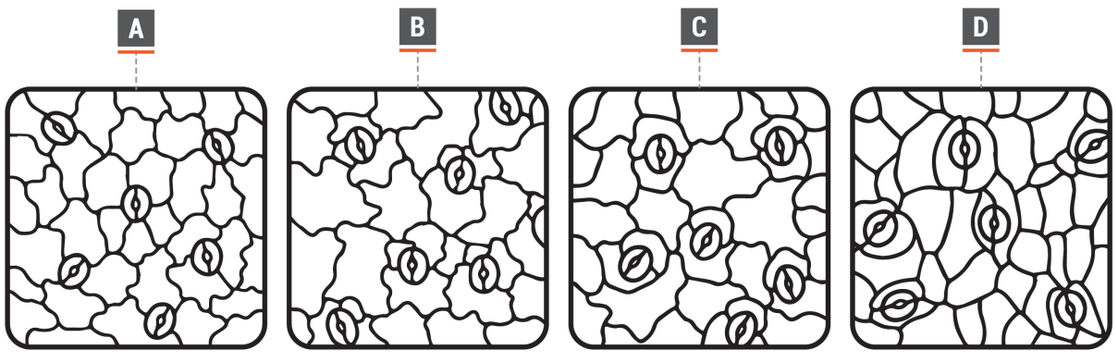

Figure 3.5. *Types of Stoma*

► Determination of Stomatal Index

Place fragments of leaves, about 5×5 mm in size, in a test tube containing about 5 mL of chloral hydrate TS and heat on a water-bath for about 15 min or until the fragments are transparent. Transfer a fragment to a slide and prepare it as described earlier, the lower epidermis uppermost, in chloral hydrate TS; place a small drop of glycerol/ethanol TS at one side of the cover glass to prevent the material from drying. Examine under a microscope with a 40× objective and a 6× eyepiece, equipped with a drawing apparatus. Mark on the drawing paper a cross (x) for each epidermal cell and a circle (o) for each stoma. Calculate the stomatal index as follows:

$$stomal\ index = \frac{S \times 100}{S + E}$$

where:

- S = the number of stomata in a given area of leaf ;
- E = the number of epidermal cells (including trichomes) in the same area of leaf.

For each leaf sample, no less than 10 determinations should be performed and the average index calculated.

MICROSUBLIMATION

Mount a small, square metal plate, about 4×4 cm in size, on a square of a suitable board from which a central hole, about 1 cm in diameter, has been cut. Place a metal ring, about 1 cm in diameter and 8 mm in height, at the center of the metal plate aligned with the hole of the asbestos board. Place about 0.1–0.2 g of powdered material inside the ring to form an even layer, about 2 mm thick. Cover the ring with a clean slide. Heat gently and gradually over a small flame of a micro burner. Change the slide if a large amount of moisture or sublimate is observed. Remove the slide from the ring, set it aside until the sublimate has dried and then examine under a microscope without adding any fluid and without a cover glass. Prepare 4–5 slides in this manner.

A heating stage allows the temperature of sublimation to be recorded.

III. Tests for Herbal Drugs

<31> UNIFORMITY OF DOSAGE UNITS

The unit can refer to a dosage form that will deliver the dose required. It may be (1) a dosage form packed in single-unit containers such as bottles or (2) a dosage form packed in pre-metered dosage units such as capsules and blisters. The uniformity of dosage units can be demonstrated by either 2 methods, mass (or weight) variation or content uniformity.

Content Uniformity can be applied to coated tablets, other than film-coated tablets containing 50 mg or more of an active ingredient that comprise 50% or more (by mass) of one tablet.

When the test for Content Uniformity is not required, the test for Mass Variations may be applied for products containing 50 mg or more of the active ingredient comprising 50% or more, by mass, of the dosage unit or in case of hard capsules, the capsule content, except that uniformity of other active ingredients present in lesser proportions is demonstrated by meeting Content Uniformity.

<32> MASS (WEIGHT) VARIATION

For the determination of dosage-unit uniformity by mass variation, select not less than 30 units and proceed as follows for the dosage form designated.

UNCOATED TABLETS

Accurately weigh 10 tablets individually. From the result of the assay, obtained as directed in the individual monograph, calculate the content of the active ingredient in each of the 10 tablets, assuming homogeneous distribution of the active ingredient.

HARD CAPSULES

Accurately weigh 10 capsules individually (gross mass per capsule), taking care to preserve the identity of each capsule. Remove the contents of each capsule by suitable means. Weigh accurately the emptied shells individually. Calculate the net mass of its contents for each capsule by subtracting the mass of the shell (tare) from the respective gross mass. From the results of the assay, obtained as directed in the individual monograph, calculate the content of active ingredient in each of the capsules, assuming homogenous distribution of the active ingredient.

For unit dose products, the test allows checking if the units of the same batch feature the same weight. In order to carry out the test, it is required to previously establish the weighted mean of the batch units.

<33> DELIVERABLE VOLUME

The following tests are designed to provide assurance that oral solutions and suspensions, when transferred from the original container, will deliver the volume of dosage form that is declared on the label of the article. The tests are applicable to products labeled to contain not more than 250 mL, whether supplied as liquid preparations or liquid preparations that are reconstituted from solids upon the addition of a designated volume of a specific diluent. For the determination of deliverable volume, select not fewer than 30 containers and proceed as follows for the dosage form designated.

For oral solution, oral suspension, syrup powders that are labeled to state the volume of oral solution or oral suspension that results when powder is constituted with the volume of diluent stated in the labeling, mix contents of 10 containers individually. Constitute 10 containers with the volume of diluent stated in the labeling, accurately measured and mixed.

PROCEDURE

Gently pour the contents of each container into a separate dry graduated cylinder of a rated capacity, not exceeding two and a half times (2.5) the volume to be measured, and calibrate "to contain". Exercise care to avoid formation of bubbles and allow them to drain for a period not exceeding 30 min, in the case of multiple-unit containers, and 5 seconds in the case of single unit containers, unless otherwise specified in the individual monograph. When free from air bubbles, measure the volume of each mixture. Alternatively, in the case of products of low volume packaged in single-unit containers, compute the volume as follows. Discharge into a suitable tared container (allowing drainage for not more than 5 seconds). Determine the mass by difference and by computing the volume after determining the apparent density. The requirements are met if the average volume complies with the Acceptance Table.

Table 3.5. Acceptance Table

LEVEL	NUMBER TESTED	CRITERIA	INTERPRETATION / DISPOSITION
1	10	Average volume is not less than 100% and no container (1) is less than 95%, or (2) more than 110% for single-unit containers.	Meets requirements for deliverable volume.
		1) If average volume is less than 100% of the label claim but no container is less than 95% of the labeled amount, or 2) If volume of not more than 1 container is less than 95% but not less than 90% of the declared amount or is more than 110% for single-unit container	Perform the test on 20 additional containers.
2	30	Average volume in not less than 100% of the amount declared in the labeling and volume from not more than 1 of the 30 containers is: 1) Less than 90% of that declared in the labeling, or 2) More than 110% but not more than 115% of that declared on the labeling for single-unit containers.	Meets requirements for deliverable volume.

<34> pH

For compendial purposes, pH is defined as the value given by a suitable, properly calibrated, potentiometric sensor and measuring system. [Note—The measuring system has traditionally been referred to as the "pH meter." While the pH meter is still in common use, the measuring system can also be embedded inside the pH sensor, and the pH signal can be transmitted digitally to an external device such as a computer, Programmable Logic Controller (PLC), Distributed Control System (DCS), data acquisition system, terminal, or other microprocessor-controlled device.] By definition, pH is equal to $-\log_{10}[a_H^+]$ where a_H^+ is the activity of the hydrogen (H^+) or hydronium ion (H_3O^+), and the hydrogen ion activity very closely approximates the hydrogen ion concentration.

The practical pH scale is defined:

$$pH = pH_s + [(E - E_s) / k]$$

- E = measured potential where the galvanic cell contains the solution under test (pH)
- E_s = measured potential where the galvanic cell contains the appropriate buffer solution for calibration (pHS)
- k = change in potential/unit change in pH and is derived from the Nernst equation (as follows)

$$k = \log_e(10) \times (RT / nF)$$

- R = 8.314 **J**/mole/°K
- T = temperature (°K)
- n = moles/half-reaction
- F = Faraday constant, 96485 **C**/mole

The resulting equation is [0.05916 + 0.0001984(T − 25°)] volts at temperature T.

Values of k from 15°–35° are provided in ***Table 3.6.***

Table 3.6. Values of k for Various Temperatures

TEMPERATURE (°c)	k (V)
15.00	0.05718
20.00	0.05817
25.00	0.05916
30.00	0.06016
35.00	0.06115

Values of k at other temperatures can be determined from the equation above. For practical purposes, values of k are determined from pH sensor calibration.

pH MEASUREMENT SYSTEM

The measurement system consists of:

1. A measuring electrode sensitive to hydrogen-ion activity, typically a glass electrode, though other electrode types are possible.

2. A suitable reference electrode, for example, a silver–silver chloride electrode.

3. A voltage measurement system with an input resistance capable of measuring at a high input impedance of the pH sensor. The measuring and reference electrode may be separated or combined. The voltage measurement system may be separated from the pH sensor or integrated into the sensor. For most applications, a temperature measurement will be necessary for compensation of the Nernst temperature influence described above. A temperature device may be embedded into the pH sensor, or an external temperature device may be used.

INSTRUMENT REQUIREMENTS

The measurement system shall be capable of performing a two-point (or more) pH calibration as discussed below. The accuracy of the pH measurement system is described in the *Calibration* section. The resolution of the pH measurement system shall be at least 0.01 pH. The instrument shall be capable of temperature-compensating the pH sensor measurement to convert the millivolt signal to pH units at any temperature, either automatically using a temperature device built into the sensor system or by manual entry of the sample temperature into the measurement system. The accuracy of the temperature measurement system shall be ±1°. The resolution of the temperature measurement system shall be at least 0.1°.

Lab-based pH measurements are typically performed at 25 ± 2° unless otherwise specified in the individual monograph or herein. However, temperatures outside this range are acceptable if samples are more conveniently prepared at alternative temperatures. Examples of non-lab-based measurements include test samples inside process pipes, vessels, tanks, and other non-standard processing conditions.

[Note—The definitions of pH, the pH scale, and the values assigned to the buffer solutions for calibration are for the purpose of establishing a practical, operational system so that results may be compared between laboratories. The measured pH values do not necessarily correspond exactly to those obtained by the definition, $pH = -\log_{10}[a_H^+]$, rather the values obtained are closely related to the activity of the hydrogen-ion in aqueous solutions.]

[Note—Where a pH measurement system is calibrated by use of an aqueous buffer and then used to measure the pH of nonaqueous systems, the ionization constant of the acid or base, the dielectric constant of the medium, the liquid-junction potential (which may give rise to errors of approximately 1 pH unit), and the hydrogen-ion response of the glass electrode are all changed. For these reasons, the values so obtained with solutions that are only partially aqueous in character can be regarded only as apparent pH values.]

BUFFER SOLUTIONS FOR CALIBRATION OF THE pH MEASUREMENT SYSTEM

Buffer solutions for calibration are prepared as directed in ***Table 3.7***. Buffer salts of requisite purity can be obtained from the National Institute of Standards and Technology (NIST), other national authorities, or other suppliers. Buffer solutions should be stored in appropriate containers that ensure stability of the pH through the expiry date, and fitted with a tight closure.

For buffer solutions greater than 11, the storage should be in containers that are resistant to or reduce carbon dioxide intrusion, which would lower the pH of the buffer. For buffer solutions lower than 11, they should typically be prepared at intervals not to exceed 3 months. For buffer solutions greater than 11, they should typically be prepared and used fresh unless carbon dioxide ingress is restricted. All buffer solutions should be prepared using purified water.

Table 3.7 indicates the pH of the buffer solutions as a function of temperature. The instructions presented here are intended for the preparation of solutions having the designated molal (m) concentrations. However, in order to facilitate their preparation, the instructions are given in molarity. The difference in concentration between molality and molarity preparations for these buffer solutions is less than 1%, and the pH difference is negligible. Calibration using buffer solutions shall be done in the temperature range of the buffers listed in ***Table 3.7***.

[Note—The Nernst temperature compensation corrects only for the electrode millivolt output change with temperature, not the actual pH change of the buffer solution with temperature, which is unique for each buffer.] Features such as automatic buffer recognition or buffer pH–temperature correction are available for convenience in accommodating the temperature influence on buffer solutions. The pH–temperature response can be determined from the values in ***Table 3.7.***

Table 3.7. pH Values of Buffer Solutions for Calibration

Temperature (°C)	Potassium Tetra-oxalate, 0.05 m	Potassium Hydrogen Tartrate, Saturated at 25°	Potassium Dihydrogen Citrate, 0.05 M	Potassium Biphthalate, 0.05 m	Equimolal Phosphate, 0.05 m	Potassium Dihydrogen Phosphate, 0.0087 M and Disodium Hydrogen Phosphate, 0.0303 M	Sodium Tetraborate, 0.01 m	Sodium Tetraborate, 0.01 m, Sodium Carbonate, 0.025 M and Sodium carbonate, 0.025 M	Calcium Hydroxide, Saturated at 25°
10	1.67	—	—	4.00	6.92	—	9.33	—	13.00
15	1.67	—	3.80	4.00	6.90	7.45	9.28	10.12	12.81
20	1.68	—	3.79	4.00	6.88	7.43	9.23	10.06	12.63
25	1.68	3.56	3.78	4.01	6.86	7.41	9.18	10.01	12.45
30	1.68	3.55	3.77	4.02	6.85	7.40	9.14	9.97	12.29
35	1.69	3.55	3.76	4.02	6.84	7.39	9.10	9.93	12.13
40	1.69	—	—	4.04	6.84	—	9.07	—	11.98
45	1.70	—	—	4.05	6.83	—	9.04	—	11.84
50	1.71	—	—	4.06	6.83	—	9.01	—	11.71
55	1.72	—	—	4.08	6.83	—	8.99	—	11.57
60	1.72	—	—	4.09	6.84	—	8.96	—	11.45
ΔpH/Δ°C	0.0010	−0.0014	−0.0022	0.0018	−0.0016	−0.0028	−0.0074	−0.0096	−0.0310

Preparation of alternative volumes at the same concentrations to those indicated below is acceptable.

Potassium tetraoxalate, 0.05 m: Dissolve 12.61 g of $KH3(C_2O_4)_2 \cdot 2H_2O$, and dilute with water to make 1000 mL.

Potassium hydrogen tartrate, saturated at 25°: Add $C_4H_5KO_6$ to water until saturation is exceeded at 25°. Then, filter or decant.

Potassium dihydrogen citrate, 0.05 M: Dissolve 11.41 g of $C_6H_7KO_4$, and dilute with water to make 1000 mL.

Potassium biphthalate, 0.05 m: Dissolve 10.12 g of $KHC_8H_4O_4$, previously dried at 110° for 1 hour, and dilute with water to make 1000 mL.

Equimolal phosphate, 0.05 m: Dissolve 3.53 g of disodium hydrogen phosphate (Na_2HPO_4) and 3.39 g of potassium dihydrogen phosphate (KH_2PO_4), each previously dried at 120° for 2 hours, and dilute with water to make 1000 mL.

Potassium dihydrogen phosphate, 0.0087 M and **disodium hydrogen phosphate, 0.0303 M:** Dissolve 1.18 g of KH_2PO_4 and 4.3 g of Na_2HPO_4, both dried for 2 hours at 120 ± 2°, and dilute with water to make 1000 mL.

Sodium tetraborate, 0.01 m: Dissolve 3.8 g of $Na_2B_4O_7 \cdot 10H_2O$, and dilute with water to make 1000 mL. Protect from absorption of carbon dioxide.

Sodium carbonate, 0.025 M and **sodium bicarbonate, 0.025 M:** Dissolve 2.64 g of sodium carbonate (Na_2CO_3) and 2.09 g of sodium bicarbonate ($NaHCO_3$), and dilute with water to make 1000 mL.

Calcium hydroxide, saturated at 25°: Add $Ca(OH)_2$ to water until saturation is exceeded at 25°. Use water that has been recently boiled and protected from the atmosphere to limit carbon dioxide absorption. Then, filter or decant.

<35> VISCOSITY

NEWTONIAN VISCOSITY

If an external stress is exerted on a fluid, the latter will flow to a degree that is determined by internal friction forces derived from internal molecular interactions and by the magnitude of the external stress applied. The measure of resistance to flow is defined as the viscosity of the fluid. The coefficient of viscosity, η, historically termed the absolute viscosity, is defined by Newton's Law of Viscous Flow:

$$\sigma = \eta \bullet \dot{\gamma} \quad (1)$$

- σ = Shear stress, defined as the applied stress that causes successive parallel layers of a material body to move in the own planes relative to each other
- $\dot{\gamma}$ = Shear rate, defined as the rate of change of shear strain with time

It should be noted that although stress and strain are often used interchangeably, the terms are not used interchangeably in rheology: stress is synonymous with force per unit area or a system of forces per unit area, and strain refers to deformation (i.e., a change in shape or size). A fluid is said to exhibit Newtonian flow if the viscosity is a constant that is independent of the shear rate or applied shear stress. Because viscosity depends on temperature, the temperature of the substance being measured should be controlled to within ±0.1°.

In general, Newton's law is obeyed by liquids of low molecular weight and by solutions of low molecular weight solutes in low molecular weight solvents. However, liquids of high molecular weight, solutions that contain high molecular weight solutes and colloidal dispersions (e.g., suspensions and emulsions) typically do not obey Newton's Law of Viscous Flow and are referred to as non-Newtonian fluids.

In the International System of Units (SI), the unit of viscosity is the pascal-second ($Pa \cdot s$), which is equivalent to 1 $kg \cdot m^{-1} \cdot s^{-1}$ or 10 Poise in the centimeter-gram-second (cgs) system. The viscosities of most Newtonian fluids are measured in millipascal-seconds (1 $Pa \cdot s$ = 1000 $mPa \cdot s$; 1 $mPa \cdot s$ = 1 centipoise). Kinematic viscosity, ν, relates the viscous force to the inertial force by relating the Newtonian viscosity of a fluid to its density, ρ, at the same temperature:

$$v = \frac{\eta}{\rho} \qquad (2)$$

The units of kinematic viscosity in SI units are $m^2 \cdot s^{-1}$ or, more commonly, $mm^2 \cdot s^{-1}$. [Note^{-1} mm2 $\cdot$ s^{-1} = 1 centistoke (cS or cSt). Stokes or centistokes are the units in the cgs system for kinematic viscosity.]

MEASUREMENT OF NEWTONIAN VISCOSITY USING A CAPILLARY VISCOMETER

The viscosity of a Newtonian fluid is measured by the use of glass capillary viscometers, examples of which are shown in ***Figure 3.6*** (simple U-tube capillary viscometer, also referred to as an Ostwald-type capillary viscometer) and ***Figure 3.7*** (constant or suspended-level viscometer, also referred to as an Ubbelohde-type capillary viscometer).

[Note—When the term viscosity is used herein, it refers to absolute or Newtonian viscosity.] The constant- or suspended-level viscometer is a more robust design that is less influenced by temperature variations.

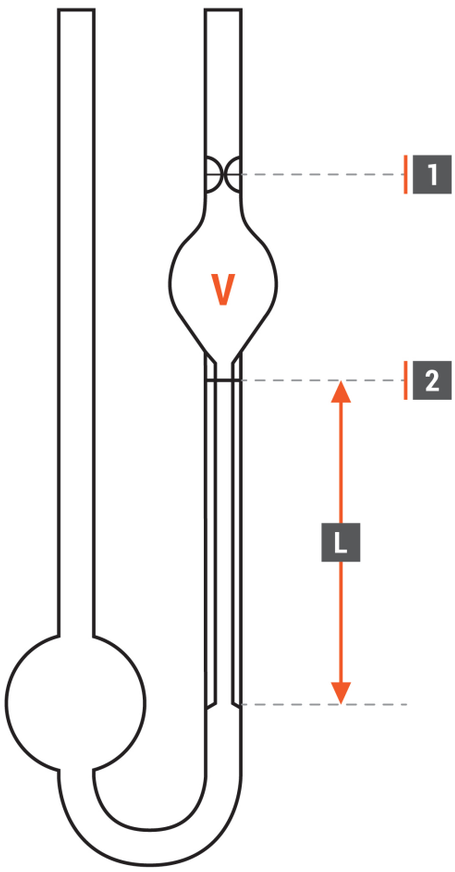

Figure 3.6. Simple U-tube (or Ostwald-type) Capillary Viscometer.
[Note—Variables are defined in the text.]

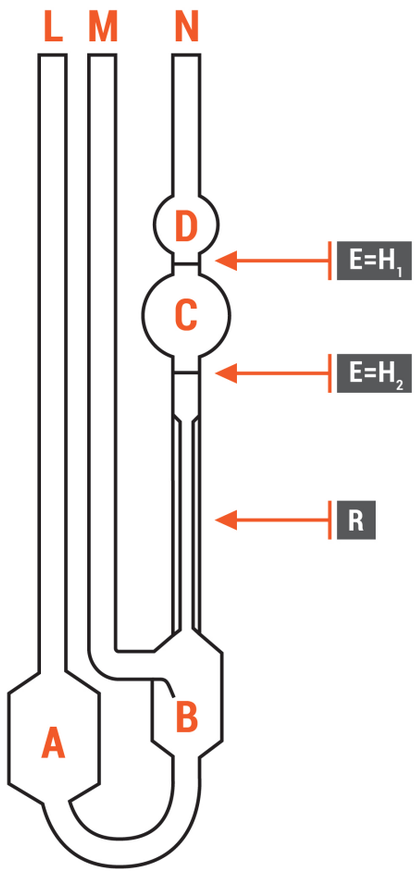

Figure 3.7. *Suspended-level (or Ubbelohde-type) Capillary Viscometer.*
[Note—Variables are defined in Viscosity—Capillary Methods 911.]

The following example is based on ***Figure 3.6***, and the same principle is applicable to ***Figure 3.7***. With the apparatus in Figure 1, the flow time, t, is measured for a given volume of fluid, V, as defined between lines 1 and 2 of Figure 1, to flow through a capillary tube having a length equal to L and a radius equal to r. As long as the density of the fluid, ρ, is known, the viscosity can be calculated using the Poiseuille equation:

$$\eta = \frac{\pi r^4 t \Delta P}{8 L V} \quad (3)$$

- P = pressure

The applied pressure differential, ΔP, is defined as:

$$\Delta P = (h_1 - h_2) \times g \times \rho \quad (4)$$

- h_1 = height of the fluid in the tube at line 1 measured from the bottom position
- h_2 = height of the fluid in the tube at line 2 measured from the bottom position
- g = gravitational acceleration (standard value = 9.80665 m · s−2)

Glass capillary viscometers generally are categorized as low shear stress instruments because ΔP is relatively small. The typical ranges for shear stress, σ, and shear rate, $\dot{\gamma}$ (at the wall), are 0.1–15 Pa and 100–20,000 s^{-1}, respectively.

The absolute dimensions of a capillary tube usually are too difficult to measure conveniently. As a result, an empirical calibration is employed for each viscometer tube. Collecting all the constants in Equations 3 and 4 into a single viscometer constant, k, yields the approximate equation for kinematic viscosity:

$$v = k \times t \quad (5)$$

To determine k, analysts measure the flow time for a liquid of known viscosity at a given temperature. This result is used to calculate the viscometer constant for that particular temperature.

In work requiring a high degree of accuracy, analysts can use the following equation:

$$v = k \times t - (E/t^2) \quad (6)$$

- E = kinetic energy factor, in mm2 · s

The viscometer constant, k, and kinetic factor, E, are determined from flow times measured for a set of stable, clean, Newtonian fluids of known kinematic viscosity (viscosity standard reference materials).

Once the viscometer constant(s) have been determined for a given temperature by the use of appropriate viscosity standards, the values can be used until the viscometer is subjected to thermal or mechanical stress that alters the

dimensions of the capillary.

Generally, calibration, operation, and cleaning of viscometers should be performed according to instrument manufacturer's recommendations. Capillary viscometers must be scrupulously clean and should display no residual adsorbed or particulate organic or inorganic contamination. Surface contaminants may alter the dimensions of the capillary and the interfacial tension.

The procedures detailed in *Viscosity—Capillary Methods 911* are intended for use with fluids that exhibit Newtonian behavior when subjected to shear stress. These procedures are not suitable for the characterization of non-Newtonian fluids because they may give erroneous and variable results if used with such fluids.

Capillary viscometers described in 911 typically measure rheological properties at only one shear rate during one pass of the sample through the capillary. Because of the nonlinearity of the shear rate–shear stress relationship for non-Newtonian fluids, this measurement is inadequate unless provisions are made for flow measurements at multiple rates of shear. Even more problematic for non-Newtonian fluids are the decrease in shear rate from the capillary wall to the center of the capillary and the variation in shear stress with time as the liquid level in the capillary viscometer decreases. Thus, capillary viscometers are inherently unsuitable for use in characterizing non-Newtonian fluids.

Because of the small ΔP attained with glass capillary viscometers, their use in measurements of high-viscosity fluids is impractical. Capillary-extrusion viscometers that use an external pressurized gas reservoir or other source of constant pressure are the only practical capillary instrumental alternative in these instances. In addition, capillary-extrusion viscometers may allow the operator to vary the shear rate or stress, thus enabling the characterization of non-Newtonian fluids. Chapter 911 does not address such devices.

MEASUREMENT OF NEWTONIAN VISCOSITY USING A ROTATIONAL VISCOMETER

The principle of the method is to measure the force (torque) acting on a rotor when it rotates at a constant angular velocity (rotational speed) in a liquid. Rotational viscometers are used for measuring the viscosity of Newtonian fluids or the apparent viscosity of non-Newtonian fluids. Detailed methods and procedures for rotational instruments are provided in *Viscosity—Rotational Methods 912*. Measurement of Newtonian viscosity can be performed on rotational viscometers/rheometers following the methods and procedures proposed in *Viscosity—Rotational Methods 912*. The calculated viscosity of Newtonian fluids should be the same (within experimental error), regardless of the rate of shear (or rotational speed).

NON-NEWTONIAN RHEOLOGY

Newton's Law Of Viscous Deformation or Flow, described in the section on *Newtonian Viscosity,* describes the relationship between an applied stress, σ, and the resultant flow with a rate of shear, or velocity gradient, of γ. Disperse systems (i.e., suspensions or emulsions) or fluids that contain macromolecular components usually do not obey Newton's law. These exceptions to Newtonian behavior are the subject of this section. The categorization of such exceptions into different flow behaviors such as shear-thinning (pseudoplastic), shear-thickening (dilatant), Bingham, etc., is not rigid. Non-Newtonian fluids may exhibit different rheological behavior depending on shear rate, shear stress, and temperature. Time-dependent rheological behavior, such as rheopexy or thixotropy, is not considered in this chapter.

The rheological behavior of a non-Newtonian fluid is evident when shear stress is plotted as a function of shear rate. The shear stress–shear rate relationship for Newtonian compositions is linear and passes through the origin, whereas the shear stress–shear rate relationship for non-Newtonian fluids is nonlinear and may not pass through the origin, or it is linear but does not pass through the origin (Bingham body). Rheograms or flow curves, of some typical non-Newtonian fluids are contrasted with those of Newtonian fluids in ***Figure 3.8.***

All the rheograms in ***Figure 3.8*** can be described using the Herschel–Bulkley equation:

$$\sigma = K \cdot \dot{\gamma}^{n} + \sigma_0 \quad (7)$$

- σ = shear stress, in Pa
- K = consistency coefficient
- $\dot{\gamma}$ = shear rate, in s^{-1}
- n = the flow behavior index
- σ_0 = yield stress, in Pa

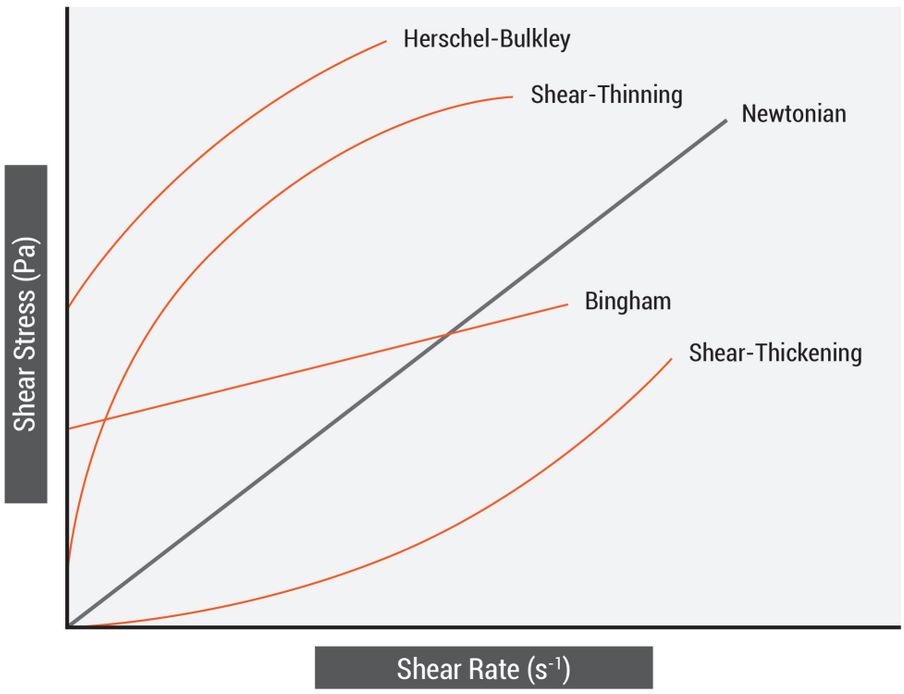

Figure 3.8. Rheograms of Newtonian and Typical Time-independent Non-Newtonian Fluids.

For Newtonian fluids and Bingham plastics, K is designated as the viscosity (η) and plastic viscosity (ηpl), respectively. Because the viscosity of Newtonian fluids and the plastic viscosity of Bingham plastics are independent of the shear stress or shear rate, the flow behavior index, n, is equal to 1 for Newtonian fluids and Bingham plastics. For shear-thinning fluids, 0 < n < 1, and for shear-thickening fluids, 1 < n < ∞. Shear thinning can be observed in certain complex solutions such as aqueous hydrocolloid solutions. Dilatant behavior is generally exhibited by concentrated suspensions of non-aggregating monodisperse solid particles such as mixtures of corn starch and water. At very high viscosities or very low shear stresses, Bingham plastics exhibit a yield stress, σ0, a shear stress below which no apparent deformation occurs (***Figure 3.8***). Other non-Newtonian fluids do not appear to exhibit a yield stress, σ0, because flow occurs even at very low shear stresses. Thus, for non-Bingham bodies, in the absence of σ0 the Herschel–Bulkley equation reduces to the Power Law, i.e., the Ostwald–de Waele equation:

$$\sigma = K \cdot \dot{\gamma}^{n} \quad (8)$$

Many pharmaceutical macromolecular materials exhibit non-Newtonian behavior in solution. Although the viscosity of

Newtonian fluids is a constant, i.e., $\eta = \sigma/\gamma$ so that η is independent of shear rate or shear stress, there is no singular value of viscosity for non-Newtonian fluids because the viscosity of non-Newtonian fluids varies with shear rate or shear stress. Accordingly, applying the Newtonian concept of viscosity to non-Newtonian fluids is inappropriate. The viscosity value observed for a non-Newtonian fluid is an apparent viscosity, η_{ap}p, and is a function of the conditions of measurement. As the shear rate or shear stress changes, the apparent viscosity is likely to change as well. ***Figure 3.9*** depicts the dependence of η_{app} on the shear rate for an aqueous solution of a typical hydrocolloid.

Figure 3.9 depicts apparent viscosities, η_{app}, ranging from high values at very low rates of shear to low values at very high rates of shear. The curve's asymptotes—corresponding to the zero-shear viscosity (η_0) and the infinite-shear viscosity (η_∞)—together with the linear log–log region at intermediate rates of shear, are characteristic of the shear-thinning behavior of these materials.

During the course of manufacturing, in-process materials that exhibit non-Newtonian behavior may be subjected to wide ranges of shear rates and shear stresses. Fluid dynamic conditions during drug product storage and administration and release of the active constituent(s) could broaden those ranges even further. For materials that exhibit non-Newtonian behavior in a solution or dispersion, the rheological profile (rheogram or flow curve), as described in ***Figure 3.8***, gives a better functional characterization than a single viscosity measurement of the apparent viscosity at a single shear rate.

Determination of the rheological profiles of non-Newtonian solutions or dispersions requires the use of a rheometer or a viscometer that is capable of measuring rheological behavior over the range of shear rates and/or shear stresses, encompassing the conditions that the excipient or product is likely to encounter. The exact mathematical characterization

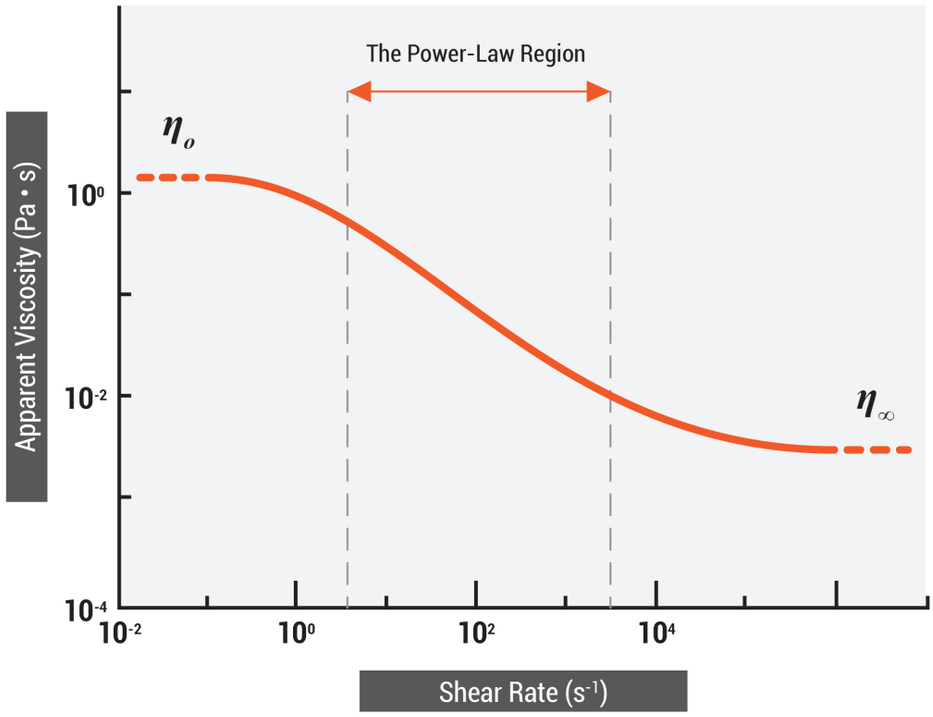

Figure 3.9. *Log–log Plot of the Dependence of η_{app} on the Shear Rate for an Aqueous Solution of a Typical Hydrocolloid.*

of deformation and flow of a test substance requires the precise delineation of sample dimensions, the forces exerted, and the resulting deformation or flow. Instruments that enable a thorough, geometrically accurate analysis of deformation and flow are termed absolute rheometers. Such absolute rheometers are preferred for measurements of non-Newtonian fluids so that results can be compared from instrument to instrument for corresponding ranges of shear stresses and shear rates. When flow or deformation is not well defined or is indeterminate, the resulting measurements are likely to correspond,

at best, to approximations of the true rheological behavior of the sample. Rheometers that can provide useful information about material behavior, but do not enable the true rheological character of the material to be determined, are known as relative rheometers. Such instruments are subject to geometry-dependent limitations. Results obtained with relative rheometers are generally not comparable to those obtained with absolute rheometers or other differently-configured relative rheometers. Relative rheometers are often called viscometers. However, the term viscometer should be reserved for a subset of rheometers that measure only steady shear flow and the corresponding Newtonian viscosity.

Temperature control during the course of measurement is critical to the rheological evaluation of non-Newtonian fluids. An Arrhenius-type equation has often been invoked to characterize Newtonian and non-Newtonian flow properties as a function of temperature.

$$\frac{\eta}{\eta_{ref}} = A \cdot e^{\left(\frac{E_0}{R}\left(\frac{1}{T} - \frac{1}{T_{ref}}\right)\right)} \qquad (9)$$

- η = Newtonian or non-Newtonian viscosity at some absolute temperature T
- η_{ref} = Newtonian or non-Newtonian viscosity at some reference temperature Tref
- A = constant
- E_0 = energy for viscous flow
- R = universal gas constant

Energies for viscous flow and the constant A are determined from experimental data. Energy dissipation by viscous flow is a function of measuring system geometry, temperature control, shear rate, and shear stress, but increased variability is observed, in particular, at high shear rates and for fluids that exhibit high apparent viscosity. Sample temperatures of non-Newtonian fluids should be controlled to ±0.1°, as is the case for Newtonian viscosity measurements.

MEASUREMENT OF VISCOSITY USING A ROTATIONAL RHEOMETER

Rotational rheometers represent a more useful alternative to most capillary viscometers for measuring the rheological characteristics of non-Newtonian fluids as long as variations in shear rate, sample temperature, and edge or end effects can be minimized. The most common absolute rotational rheometer designs include concentric (coaxial) cylinder, cone-and-plate, and parallel-plate instruments. All of these are available in models that can provide absolute rheological data—that is, data that do not require substantial corrections for end or edge effects, viscous heating, or variations in shear rate or shear stress in the sample being evaluated. Rotating spindle rheometers—often described as viscometers—are relative rheometers that typically are used to assess apparent viscosity.

An additional factor with all rheometers/viscometers that must be taken into consideration, particularly with multiphase materials, is the potential formation of a thin, less-viscous layer of fluid at the wall of the instrument. When wall effects or wall slips occur, instrument measurements may be misleading because of the actual rheological behavior of the sample. Identification of wall effects is instrument-specific. One useful method entails the comparison of results with a roughened vs. a smooth rotor. Instrument manufacturers generally provide recommendations for identification of and correction for wall slip.

Generally, calibration, operation, and cleaning of rheometers should be performed according to the instrument manufacturer's recommendations.

General chapter *Viscosity—Rotational Methods 912* describes the procedures for the most common types of rheometers:

spindle rheometers (viscometers) and concentric (coaxial) cylinder, cone-and-plate, and parallel-plate (parallel disk) instruments.

CALCULATION OF SHEAR RATE, SHEAR STRESS, AND VISCOSITY USING A CONCENTRIC (COAXIAL) CYLINDER RHEOMETER

For non-Newtonian liquids, it is essential to specify the shear stress, σ, or the shear rate, $\dot{\gamma}$, at which the viscosity is measured. Under narrow gap conditions (conditions satisfied in absolute rheometers), the shear rate $\dot{\gamma}$ in s^{-1}, and the shear stress σ, in Pa ($N \cdot m^{-2}$ or $kg \cdot m^{-1} \cdot s^{-2}$), are calculated using the following equations:

$$\dot{\gamma} = \left(\frac{R_O^2 + R_I^2}{R_O^2 - R_I^2} \right) \omega \quad (10)$$

$$\sigma = \left(\frac{1}{4\pi h R_O^2} + \frac{1}{4\pi h R_I^2} \right) M \quad (11)$$

- w = radius of the outer cylinder (m)
- R_I = radius of the inner cylinder (m)
- ω = angular velocity (radian/s)
- M = torque acting on the cylinder surface ($N \cdot m$)
- h = height of immersion of the inner cylinder in the liquid medium (m)

Generally, the angular viscosity can be calculated using the equation:

$$\omega = \left(\frac{2\pi}{60} \right) n \quad (12)$$

- n = otational speed, in revolutions per min (rpm)

For laminar flow, the viscosity η (or apparent viscosity η_{App}), in $Pa \cdot s$, is determined using the following equation: [Note—1 $Pa \cdot s$ = 1000 $mPa \cdot s$.]

$$\eta \text{ or } \eta_{app} = \frac{1}{4\pi h} \left(\frac{1}{R_I^2} + \frac{1}{R_O^2} \right) \frac{M}{\omega} = k \frac{M}{\omega} \quad (13)$$

- k = constant of the apparatus (radians/m3)

CALCULATION OF SHEAR RATE, SHEAR STRESS, AND VISCOSITY USING A CONE-AND-PLATE RHEOMETER

The shear rate γ in s−1, and the shear stress σ, in Pa, are calculated using the following equations:

$$\dot{\gamma} = \left(\frac{1}{\alpha} \right) M \qquad (14)$$

$$\sigma = \left(\frac{1}{\frac{2}{3} \pi R^3} \right) M \qquad (15)$$

- α = angle between the flat plate and the cone (radians)
- R = radius of the cone (m)
- ω = angular velocity (radian/s)
- W = torque acting on the cylinder surface (N · m)

For laminar flow, the viscosity η (or apparent viscosity η_{App}), in Pa · s, is determined using this equation:

$$\eta \text{ or } \eta_{app} = \left(\frac{3\alpha}{2\pi R^3} \right) \left(\frac{M}{\omega} \right) = k \frac{M}{\omega} \qquad (16)$$

- k = constant of the apparatus (radians/m3)

MEASUREMENT OF RHEOLOGICAL PROPERTIES USING A NONROTATIONAL RHEOMETER

Alternative nonrotational rheometer procedures for measuring rheological properties of non-Newtonian fluids include slit rheometers, small- and large-amplitude oscillatory shear-based rheometers, and capillary-breakup elongational rheometers. Equipment manufacturers generally provide detailed procedures, and these instruments and methods may be appropriate for use after users complete characterization and validation studies for both the equipment and the material under test. These types of rheometers are not described in *Viscosity—Rotational Methods 912*, as these are not rotational rheometers.

Reference

USP/NF 2022 Issue 1

<36> TEST FOR HARDNESS

TABLET BREAKING FORCE

There are a variety of presentations for tablets as delivery systems for pharmaceutical agents, such as rapidly disintegrating, slowly disintegrating, eroding, and chewable. The concepts of this chapter are also applicable to lozenges. Each of these presentations places a certain demand on the bonding, structure, and integrity of the compressed matrix. Tablets must be able to withstand the rigors of handling and transportation experienced in the manufacturing plant, in the drug distribution system, and in the field at the hands of the end users (patients/consumers). Manufacturing processes such as coating, packaging, and printing can involve considerable stresses, which the tablets must be able to withstand. For these reasons, several tests are available to assess the strength of the compact as well as the response to contact with water and other liquids. These tests include disintegration, dissolution, tablet friability and tablet breaking force, which are the subject of this chapter. The mechanical strength of tablets is of considerable importance and is routinely measured. Tablet strength serves both as a criterion by which to guide product development and as a quality control specification. An acceptable value for the strength of the compact must consider the intended use as well as the mechanism of release of the active ingredient(s) from the dosage form.

One commonly employed test on the ability of tablets to withstand mechanical stresses determines their resistance to chipping and surface abrasion by tumbling them in a rotating cylinder. The percentage weight loss after tumbling is referred to as the friability of the tablets. In addition to the loss of active drug, chipping and abrasion can each have a significant impact upon the success of subsequent manufacturing operations such as coating and packaging, and impact the consumer's perceived elegance of the dosage form.

Another measure of the mechanical integrity of tablets is their “breaking force,” which is the force required to produce failure (i.e., breakage) in a specific plane. The tablets are generally placed between two platens, one of which moves to apply sufficient force to the tablet to cause fracture. For conventional, round (circular cross-section) tablets, loading occurs across their diameter (sometimes referred to as diametral loading), and fracture occurs in that plane.

As previously stated, friability and breaking force measure different aspects of the compact strength. Depending upon the formulation, manufacturing method, and intended use, the results from one of the tests may be a better indicator of the quality of the dosage form than the other. This determination is best made at the time the formulation and manufacturing process are defined.

The breaking force of tablets is commonly called “hardness” in the pharmaceutical literature. However, the use of this term is misleading. In material science, the term “hardness” refers to the resistance of a surface to penetration or indentation by a small probe. The term “crushing strength” is also frequently used to describe the resistance of tablets to the application of a compressive load. Although this term describes the true nature of the test more accurately than “hardness” does, it implies that tablets are actually crushed during the test, which often is not the case nor it is the intent of the breaking force determination. Moreover, the term “strength” in this application can be questioned, because in the physical sciences that term is often used to describe a stress (e.g., tensile strength). Thus, the term “breaking force” is preferred and will be used in the present discussion. During manufacturing of compressed tablets, decisions must be made regarding tablet weight, thickness, friability, and breaking strength targets. Control of tablet weights must be given priority because weight control directly correlates with dosing accuracy. Once the desired weight is achieved, decisions must be made concerning the relative importance of the thickness, loss on drying, friability, and breaking strength. While thickness may be viewed as a physical parameter that only influences the appearance of the tablet, it also determines the pore volume of the compact. While thickness, friability, and breaking strength are related, the relationships are not clear or easily predicted. The thickness of the tablet at constant weight may not be varied without influencing the breaking strength. It is therefore important for the manufacturer to identify which parameter most closely correlates with desired product performance.

TABLET BREAKING FORCE DETERMINATION

Early measuring devices were typically hand-operated. For example, the Monsanto (or Stokes) hardness tester was based on compressing tablets between two jaws via a spring gauge and screw. In the Pfizer hardness tester, the vertically mounted tablet was squeezed in a device that resembled a pair of pliers. In the Strong-Cobb hardness tester, the breaking load was applied through the action of a small hydraulic pump that was first operated manually but was later motorized. Problems associated with these devices were related to operator variability in rates of loading and difficulties in proper setup and calibration. Modern testers employ mechanical drives, strain gauge-based load cells for force measurements, and electronic signal processing, and therefore are preferred. However, several important issues must be considered when using them for the analytical determination of breaking force. These are discussed below.

► Platens

The platens should be parallel. Their faces should be polished smooth and precision-ground perpendicularly to the direction of movement. In addition, the face of the platen should be perpendicular to the surface on which the tablet is placed to ensure the platen uniformly contacts the side of the tablet from top and bottom. Perpendicularity must be preserved during platen movement, and the mechanism should be free of any bending or torsion displacements as the load is applied. The contact faces must be larger than the area of contact with the tablet. These considerations ensure that the applied load is operating over a consistent surface area of the dosage form.

► Rate and Uniformity of Loading

Either the rate of platen movement or the rate at which the compressive force is applied (i.e., the loading rate) should be constant. Maintaining a constant loading rate avoids the rapid buildup of compressive loads, which may lead to uncontrolled crushing or shear failure and greater variability in the measured breaking force. However, constant loading rate measurements may be too slow for real-time monitoring of tablet production.

The rate at which the compressive load is applied can significantly affect results, because time-dependent processes may be involved in tablet failure. How a tablet matrix responds to differences in the loading rate depends on the mechanism of failure.

Since different formulations of tablets exhibit varying amounts of strain rate sensitivity, it is important to conduct the breaking force test in a controlled manner and recognize that instruments that operate with different platen movement or loading rates may not give comparable results for the same product.

The test must be run consistently with equipment that has been routinely calibrated. Changing from testing units of different designs or from different manufacturers will require comparison of data to ensure that the two units are subjecting the dosage form to similar stress in a similar manner. Currently available equipment provides a constant loading rate of 20 newtons (N) or less per second or a constant platen movement of 3.5 mm or less per second. Controlled and consistent breaking is an important test procedure attribute. To ensure comparability of results, testing must occur under identical conditions of loading rate or platen movement rate. Since there are certain advantages to each system of load application, both are found in practice. Because the particular testing situation and the type of tablet matrix being evaluated will pose different constraints, there is also no basis to declare an absolute preference for one system over the other. This general chapter proposes consideration of both approaches.

The different methods may lead to numerically different results for a particular tablet sample, requiring that the rate of load application or displacement must be specified along with the determined breaking force.

► Dependence of Breaking Force on Tablet Geometry and Mass

Measurements of breaking force do not take into account the dimensions or shape of the tablet. Tablet orientation and failure should occur in a manner consistent with the orientation used and failure observed during the development of the dosage form. For direct comparisons (i.e., without any normalizations of the data), breaking force measurements should be performed on tablets having the same dimensions, weights, geometry, and consistent orientation in the test

equipment.

► Tablet Orientation

Tablet orientation in diametral compression of round tablets without any scoring is unequivocal. That is, the tablet is placed between the platens so that compression occurs across a diameter. However, tablets with a unique or complex shape may have no obvious orientation for breaking force determination.

Because the breaking force for unembossed, noncircular cross-section tablets will likely depend on the tablet's orientation in the tester, it is best to settle on a standard orientation, preferably one that provides a consistent failure plane and that is readily reproduced by operators, to ensure comparability of results. In general, tablets are tested either across the diameter or parallel to the longest axis. Scored tablets have two orientation possibilities. When they are oriented with their scores perpendicular to the platen faces, the likelihood that tensile failure will occur along the scored line increases. This provides information about the strength of the matrix at the weakest point in the structure. When scored tablets are oriented with their scores parallel to the platen faces, more general information about the strength of the matrix is derived.

Capsule-shaped tablets or scored tablets may best be broken in a three-point flexure test. A fitting, which is either installed on the platens or substituted for the platens, supports the tablet at its ends and permits the breaking load to be applied to the opposite face at the unsupported midpoint of the tablet. The fittings are often available from the same source that supplies the hardness tester.

Since the orientation of the dosage form critically impacts the result of the test, it must be clearly defined in the test procedure and produce a consistent failure plane in the dosage form for the results to be comparable. The plane of failure should always be noted. If, while maintaining orientation, the plane of failure differs for a batch or sub-batch of tablets relative to previous results and direct comparison of the numerical value is not possible, this observation in itself can be an important indicator of a compression problem.

► Units, Resolution, and Calibration

Modern breaking force testers are usually calibrated in kiloponds or newtons. The relationship between these units of force (3) is 1 kilopond (kp) = 1 kilogram-force (kgf) = 9.80 newtons (N). The test results should be expressed in standard units of force which facilitate communication. Some breaking force testers will also provide a scale in Strong-Cobb units (SCU), a carryover from the days when Strong-Cobb hardness testers were in common usage. The conversion between SCU and N or kp must be viewed with caution, because the SCU is derived from a hydraulic device and is a pressure.

Generally, contemporary breaking force testers use modern electronic designs with digital readouts. Some units also have an integral printer or may be interfaced with a printer. Breaking forces should be readable to within 1 N.

Breaking force testers should be calibrated periodically. The force sensor as well as the mechanics of the apparatus needs to be considered. For the force sensor, the complete measuring range (or, at a minimum, the range used for measuring the test sample) should be calibrated to a precision of 1 N, using either the static or dynamic method. Static calibration generally employs traceable counterweights; at least three different points are checked to assess linearity. Dynamic calibration makes use of a traceable reference-load cell that is compressed between the platens. The functional calibration of a breaking force test apparatus should also confirm that the velocity and the constancy of velocity for load application or displacement are within prescribed tolerances throughout the range of platen movement.

► Sample Size

Interpretation of the breaking force data must consider not only the mean value but the consistency of the test results for multiple tablets. The range of breaking force values may provide valuable information on the consistency of the tableting process. Excessive variability in values may reflect issues with die filling, weight control, or orientation of the dosage form in the test equipment. Additionally, the breaking force result will reflect the orientation of the failure plane.

For results to be compared, the tablets must fail in the same manner throughout the test. In cases where breaking force may be particularly critical, the average plus individual breaking force values should be reported.

TENSILE STRENGTH

Tensile strength is an intensive materials property, which by definition does not depend on the size or shape of the material being measured. The measurement of tensile strength provides a more fundamental measure of the mechanical strength of the compacted material and takes into account the geometry of the tablet. The application of a vertical force (F), across the diameter of a disc-shaped tablet generates a horizontal stress (σX), which is tensile and constant along the centerline of the tablet. The value of this stress is determined using the following equation, which applies to diametral compression of cylindrical tablets.

- σX = 2F/πDH
- σX = horizontal stress
- F = breaking force
- D = tablet diameter
- H = tablet thickness

The derivation of this equation may be found in standard texts (4–6); it is based on elastic theory and the following assumptions:

- The tablet is an isotropic body. Hooke's Law is obeyed
- The modulus of elasticity in compression and in tension is the same
- Ideal point loading occurs

If the force is increased to the point of tablet fracture and if the tablet breaks into two roughly equal halves, the corresponding tensile stress at failure is historically reported as the "tensile strength." However, recent studies show that the tensile strength calculated using this method can be half or less of the actual tensile strength as measured in a three-point bend test. The discrepancy occurs because tablet fracture initiates under the intense shear stresses (8) that are present near the point of contact between the tablet and the compression platen. The intense shear stresses allow cracks to form under Mode III fracture conditions (out-of-plane shearing), even though σX is still below the strength of the compact. Once fracture initiates, the cracks propagate along the center line by Mode I fracture (opening) and at tensile stresses well below the true tensile strength of a crack-free tablet. Despite these errors the diametrical strength is still useful in many applications (9). Moreover, the data will be normalized with respect to tablet dimensions, because both diameter and thickness are included in the equation.

The derivation has been extended to convex-faced tablets:

- σX = (10F/πD2) × [(2.84H/D) − (0.126H/W) + (3.15W/D) + 0.01]−1
- σX = tensile stress
- F = breaking force
- D = tablet diameter
- H = tablet thickness
- w = central cylinder thickness (tablet wall height)

Other examples may be available in the literature.

Bending or flexure of tablets is another option for determining the tensile strength of tablets. Under ideal loading conditions, a breaking load applied to the unsupported midpoint of one face will result in the generation of pure tensile stress in the opposite face. If the tablets are right circular cylinders and are subjected to three-point flexure, the tensile strength may be estimated using the following equation:

- σX = 3FL/2H2D
- σX = tensile stress
- F = breaking force
- L = distance between supports
- H = tablet thickness
- D = tablet diameter

The value of L should be chosen judiciously to minimize errors in this calculation. The assumptions are the same as those for calculating tensile strength from diametral compression. However, tensile strengths determined by flexure and diametral compression may not agree because of likely non-ideal loading and the induction of shear failure during testing.

<37> TEST FOR FRIABILITY

This general information chapter has been harmonized with the corresponding texts of the European Pharmacopoeia and the Japanese Pharmacopoeia. The harmonized texts of these three pharmacopeias are therefore interchangeable, and the methods of the European Pharmacopoeia and/or the Japanese Pharmacopoeia may be used for demonstration of compliance instead of the present United States Pharmacopeia general information chapter method. These pharmacopeias have undertaken not to make any unilateral change to this harmonized chapter.

This chapter provides guidelines for the friability determination of compressed, uncoated tablets. The test procedure presented in this chapter is generally applicable to most compressed tablets. Measurement of tablet friability supplements other physical strength measurements, such as tablet breaking force.

Use a drum, with an internal diameter between 283 and 291 mm and a depth between 36 and 40 mm, of transparent synthetic polymer with polished internal surfaces, and subject to minimum static build-up (see figure for a typical apparatus). One side of the drum is removable. The tablets are tumbled at each turn of the drum by a curved projection with an inside radius between 75.5 and 85.5 mm that extends from the middle of the drum to the outer wall. The outer diameter of the central ring is between 24.5 and 25.5 mm. The drum is attached to the horizontal axis of a device that rotates at 25 ±1 rpm. Thus, at each turn, the tablets roll or slide and fall onto the drum wall or onto each other.

TABLET FRIABILITY APPARATUS

For tablets with a unit weight equal to or less than 650 mg, take a sample of whole tablets corresponding as near as possible to 6.5 g. For tablets with a unit weight of more than 650 mg, take a sample of 10 whole tablets. The tablets should be carefully dedusted prior to testing. Accurately weigh the tablet sample, and place the tablets in the drum. Rotate the drum 100 times, and remove the tablets. Remove any loose dust from the tablets as before, and accurately weigh.

Generally, the test is run once. If obviously cracked, cleaved, or broken tablets are present in the tablet sample after tumbling, the sample fails the test. If the results are difficult to interpret or if the weight loss is greater than the targeted value, the test should be repeated twice and the mean of the three tests determined. A maximum mean weight loss from the three samples of not more than 1.0% is considered acceptable for most products.

If tablet size or shape causes irregular tumbling, adjust the drum base so that the base forms an angle of about 10° with the horizontal and the tablets are no longer bound together when lying next to each other, which prevents them from falling freely.

Effervescent tablets and chewable tablets may have different specifications as far as friability is concerned. In the case of hygroscopic tablets, an appropriate humidity-controlled environment is required for testing. Drums with dual scooping projections, or an apparatus with more than one drum, for the running of multiple samples at one time, are also permitted.

<38> TEST FOR DISINTEGRATION

This test is provided to determine compliance with the limits on Disintegration stated in the individual monographs. This is not applicable to tablets or capsules which are intended for use as troches, or are to be chewed, or are designated as modified release dosage forms.

Determine the types of units under test from labeling and from observation, and apply the appropriate procedure to 6 or more dosage units.

For purposes of this test, disintegration does not signify a complete solution of the unit or even of its active constituents. Complete disintegration is defined as that state in which any residue of a unit, except fragments on insoluble coating or capsule shell, remaining on the screen of the apparatus is a soft mass having no palpably firm core.

GENERAL PROCEDURE

Unless otherwise specified in the individual monograph, use the procedure as directed under dosage form in ***Table 3.8***. The temperature of the immersion fluid is maintained at 37.2±2°C throughout the test. All 16 units must disintegrate completely at level 1. In all cases, if 1 or 2 units fail to disintegrate completely at level 1, repeat the test on 12 additional units. The second test is designated as level 2 with an acceptance criteria of not less than 16 of the total 18 units tested disintegrating completely.

Table 3.8. Dosage Form and Procedure

DOSAGE FORM	IMMERSION FLUID	TIME	PROCEDURE
Uncoated tablet Plain coated tablet	Water	As specified in the monograph	Place 1 tablet in each of the 6 tubes of the basket and operate the apparatus. At the end of the time limit specified, lift the basket from the fluid, observe the tablets for disintegration.
Hard gelatin capsules	Water	As specified in the monograph	Apply test as above. Attach a removable wire cloth

APPARATUS

The apparatus consists of a basket-rack assembly, a 1000-mL low-form beaker 138–160 mm in height and having an inside diameter of 97–115 mm for the immersion fluid, a thermostatic arrangement for heating the fluid 35°–39°, and a device for raising and lowering the basket in the immersion fluid at a constant frequency rate 29–32 cycles/min through a distance of NLT 53 mm and NMT 57 mm. The volume of the fluid in the vessel is such that at the highest point of the upward stroke, the wire mesh remains at least 15 mm below the surface of the fluid and descends to NLT 25 mm from the

bottom of the vessel on the downward stroke. At no time should the top of the basket-rack assembly become submerged. The time required for the upward stroke is equal to the time required for the downward stroke, and the change in stroke direction is a smooth transition, rather than an abrupt reversal of motion. The basket-rack assembly moves vertically along its axis. There is no appreciable horizontal motion or movement of the axis from the vertical.

► Basket-rack Assembly

The basket-rack assembly consists of 6 open-ended transparent tubes, each 75.0–80.0mm long and having an inside diameter of 20.7–23 mm and a wall 1.0–2.8 mm thick; the tubes are held in a vertical position by two plates, each 88–92 mm in diameter and 5–8.5 mm in thickness, with 6 holes, each 22–26 mm in diameter, equidistant from the center of the plate and equally spaced from one another. Attached to the undersurface of the lower plate is a woven stainless steel wire cloth, which has a plain square weave with 1.8–2.2-mm apertures and with a wire diameter of 0.57–0.66 mm. The parts of the apparatus are assembled and rigidly held by means of 3 bolts passing through the 2 plates. A suitable means is provided to suspend the basket-rack assembly from the raising and lowering device using a point on its axis.

The design of the basket-rack assembly may be varied somewhat, provided the specifications for the glass tubes and the screen mesh size are maintained. The basket-rack assembly conforms to the dimensions found in ***Figure 3.10.***

► Disks

The use of disks is permitted only when specified or allowed in the monograph. If specified in the individual monograph, each tube is provided with a cylindrical disk 9.35–9.65 mm thick and 20.55–20.85 mm in diameter. The disk is made of a suitable transparent plastic material having a specific gravity of 1.18–1.20. Five parallel 1.9–2.1 mm holes extend between the ends of the cylinder. One of the holes is centered on the cylindrical axis. The other holes are parallel to the cylindrical axis and centered 5.8–6.2 mm from the axis on imaginary lines perpendicular to the axis and to each other. Four identical trapezoidal-shaped planes are cut into the wall of the cylinder, nearly perpendicular to the ends of the cylinder. The trapezoidal shape is symmetrical; its parallel sides coincide with the ends of the cylinder and are parallel to an imaginary line connecting the centers of two adjacent holes 6 mm from the cylindrical axis. The parallel side of the trapezoid on the bottom of the cylinder has a length of 1.5–1.7 mm, and its bottom edges lie at a depth of 1.5–1.8 mm from the cylinder's circumference. The parallel side of the trapezoid on the top of the cylinder has a length of 9.2–9.6 mm, and its center lies at a depth of 2.5–2.7 mm from the cylinder's circumference. All surfaces of the disk are smooth. If the use of disks is specified in the individual monograph, add a disk to each tube, and operate the apparatus as directed under *Procedure*. The disks conform to dimensions found in ***Figure 3.10***.

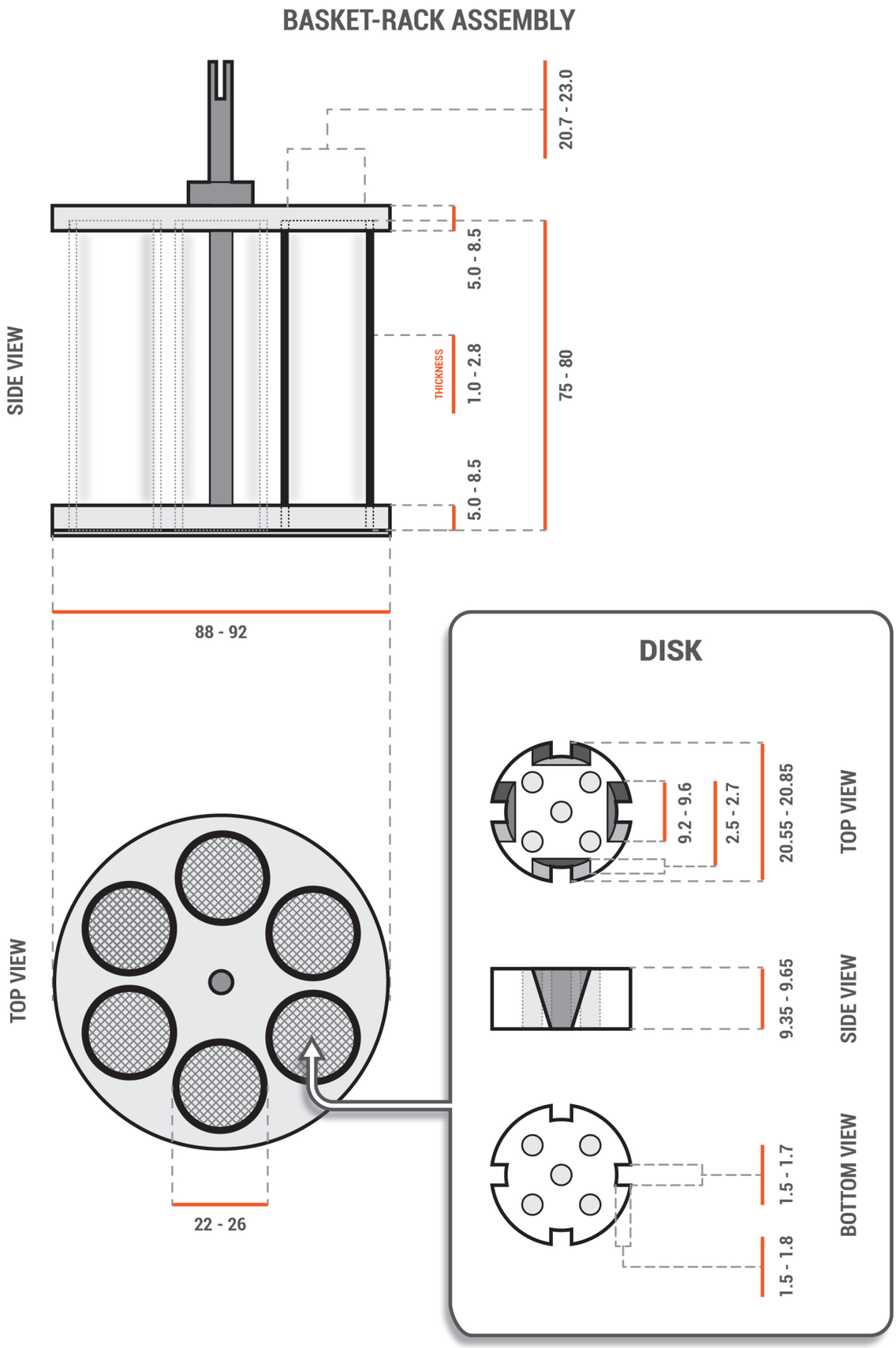

Figure 3.10. *Disintegration Apparatus (all dimensions are expressed in mm)*

IV. Methods of Analysis

<41> INSTRUMENTAL AND SPECTROSCOPIC METHODS

UV-VIS SPECTROSCOPY

Ultraviolet-visible (UV-Vis) Spectrophotometry is a method used to measure the absorbance or transmittance of light (UV or visible) by analytes as a function of their concentration.

PRINCIPLE

Spectroscopy is based on the interaction of light and chemical compounds. A substance in solution will either transmit or absorb monochromatic light passing through it.

The ratio of transmitted light to the intensity of the incident light is called transmittance and is expressed as % transmission, T. The logarithm of the inverse (reciprocal) of transmittance is the absorbance, A. When a chemical compound absorbs light, its electrons jump from a ground state of lower energy to an excited state having higher energy. The difference between the energies of the ground state and the excited state of the electrons is equal to the amount of ultraviolet or visible radiation absorbed by it producing a distinct spectrum. Absorption varies depending on the wavelength of the light being absorbed. Thus, the absorption is measured at wavelengths between 180-390 nm (UV range) or 390-800 nm (visible range). Color absorbed and observed at the visible spectrum is shown in ***Table 3.9.***

Table 3.9. The Visible Spectrum

WAVELENGTH, nm	COLOR ABSORBED	COLOR OBSERVED
390 - 420	Violet	Green-yellow
420 - 440	Violet-blue	Yellow
440 - 470	blue	Orange
470 - 500	Blue-green	Red
500 - 520	Green	Purple
520 - 550	Yellow-green	Violet
550 - 580	Yellow	Violet-blue
620 - 680	Red	Blue-green
680 - 780	Purple	-

Source: PJ Worsfold in Encyclopedia of Analytical Science, 2nd ed, 2005

Absorbance is proportional to the concentration of the absorbing substance and the length of the solution through which the light passes such that $A = \varepsilon \times c \times l$, where k or ε is the molar absorptivity, c is the concentration in moles/L at a constant length of the solution which is 1 cm. The value of A varies depending on the wavelength of light passing through the substance in solution.

UV-Vis spectrometer measurements are used for both qualitative and quantitative analyses. Qualitative analyses involve obtaining an absorption spectrum profile that allows for identification of the absorbing substance. Absorption spectrum is obtained by plotting absorbance vs wavelength to determine at which wavelength the substance absorbs maximally as well as minimally. An absorption spectrum is unique for the structure of the absorbing substance and may therefore be used to identify it. A comparison of the spectrum of the analyte or chemical compound with a specified wavelengths will allow identification of the substance or analyte.

UV-Vis spectrometer is most useful in quantitative measurements of the concentration of analytes in solution. Usually, the measurements of concentration of the absorbing analyte are done at the wavelength where the substance absorbs maximally. The absorbance measurements for compounds that absorb intensely are usually done in dilute solutions to ensure that the amount of light is significant and can be perceived by the detector. Aside from dilution, solvents used must also be transparent or non-absorbing. Thus, commonly used solvents are water, ethanol, hexane and cyclohexane.

EQUIPMENT

Two types of spectrometers are used in UV-vis analysis. The single beam spectrometer uses a fixed wavelength monochromator much like a photometer. Double beam spectrometers have control mechanism that regulates the radiation path alternately between the sample, blank and a shutter. The signal processor uses the speed of rotation of this control (a chopper or diffraction grating) to read the signal transmitted or absorbed by the blank and the sample.

Whatever the type of spectrometer used, the components of a UV-vis spectrometer consist of 1) an optical system made up of a light source that emits radiation in the range of at least 200-800 nm, a wavelength selector, the sample holder, and the detector, and 2) a signal processor that reads the output when the light of specific wavelength passes through the sample.

Below is the scheme for a double beam UV-vis spectrometer.

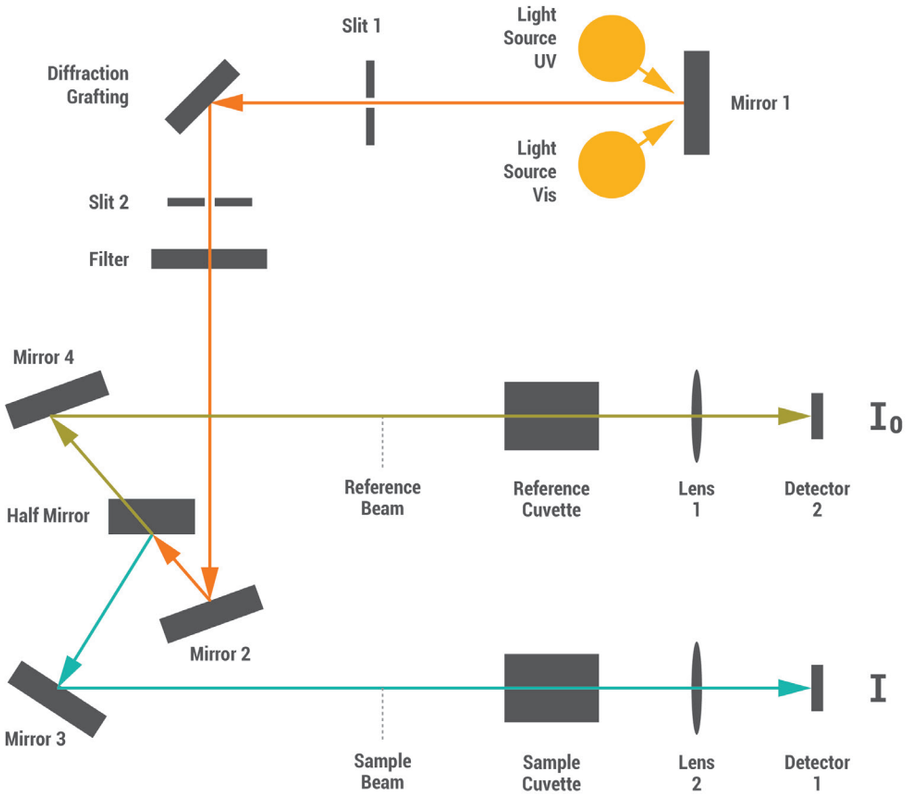

Figure 3.11. *Scheme for a Double Beam UV-Vis Spectrometer*

Figure 3.11 is a diagrammatic scheme for quantitating/ identifying absorbing compounds or analytes in a solution. The solution is contained in the sample cuvette. Source: UV-Visible Spectroscopy (msu.edu)

The light source for UV readings at wavelengths 170-375 nm is a deuterium lamp, while a tungsten lamp is used for visible readings. The latter produces light at the range 350-2,500 nm. Other spectrometers also use a halogen lamp for visible readings.

For wavelength selection, the following may be used:

1. Monochromators – separates light into a narrow band of wavelength and is dependent on the diffraction gratings, a rotating part that allows selection of desired wavelength of light.
2. Absorption filters – use colored glass or plastic that absorbs only specific wavelengths of light.
3. Interference filters or dichroic filters – made up of several layers of dielectric material; used to eliminate undesirable wavelengths by destructive interference, thereby selecting only particular wavelengths.
4. Cutoff filters – allow light below or above a certain wavelength to simply pass through and works similarly with interference filters.
5. Bandpass filters – allow a range of wavelengths to pass through and combines some functions of the cut-off filters.

Detectors can either be based on photoelectric coatings or semiconductors. The two most commonly used detectors based on semiconductor technology are photodiodes and charge-coupled devices (CCDs).

GENERAL PROCEDURE

The absorbance of light that passes through the sample is proportional to the amount of absorbing substance. Hence, its concentration. For all analyses, a reference or blank sample is used to remove absorbance due to other materials other than the component being measured. For samples using buffered aqueous solutions, the buffered aqueous solution without the substance of interest is the blank, while for bacterial cultures, the sterile culture media are the references of blank. The absorbance of the substance of interest may be plotted against a calibration curve to get its concentration. On the other hand, for very dilute samples or where matrix effects due to the presence of several components in the sample can reduce or enhance the analyte signal, the standard addition method may also be employed. The method compares the absorbance of the substance of interest and the absorbance of the sample containing varying concentrations of a known reference standard (https://www.jove.com/v/10201/method-of-standard-addition).

METHOD

1. Samples are prepared in accordance with protocols stated in the monographs. If the samples are too concentrated, these are diluted to the appropriate concentration that falls within the linear portion of the Beer-Lambert's plot of absorbance vs concentration of sample.
2. The spectrometer is turned on to allow the lamps to warm up for an appropriate period of time to stabilize them. (Refer to the spectrometer instrument manual for the appropriate time).
3. Put empty cuvettes in the cell holder. Adjust the apparatus to 0% transmittance after shutting the sample side of the light path. Then, open the shutter and adjust the instrument again to 100% transmittance or zero absorbance. After instrument adjustment, remove the cuvettes from the cell holder.
4. Select the wavelength as described in the monographs.
5. Fill a cuvette to at most ¾ its volume with the solvent used to prepare the sample. This will serve as the blank. Wipe the sides of the cuvette clean using Kimwipes or a lintless wipe. (Note: majority of plastic, disposable cuvettes are not suited for UV absorption studies because plastic generally absorbs UV light. And although glass can act as a filter that absorbs majority of UVC (100-280 nm) and UVB (280-315 nm), it however allows some UVA (315-400 nm) to pass through. Thus, the material for cuvette of choice for UV absorption determinations is quartz because quartz is transparent for majority of UV light. For certain analysis that utilizes microquantities, such as DNA and RNA analyses, cuvette-free systems are also available.)

6. Place the cuvette in the spectrometer cell holder and adjust the instrument so that the absorbance reading is zero, or transmittance is 100%.

THE ABSORPTION SPECTRUM - QUALITATIVE ANALYSIS

1. For qualitative analysis, the UV-Vis absorption spectrum is obtained by scanning the absorption of the analyte at varying wavelengths. The parameters that may be varied during the scan are the following: 1) data interval, 2 resolution (slit width), and 3) scan speed. The data interval is selected, and this is usually 1-2 selections lower than the slit width. The scan speed depends on the operator; a software calculates the total scan time.

2. Reference spectrum: The absorption spectrum of a reference standard can be compared with the sample solution. When the spectrum of the sample shows similar intensities of absorption at the same wavelength as the reference, the identity of the sample may be confirmed. A sample reference spectrum is shown in the figure below.

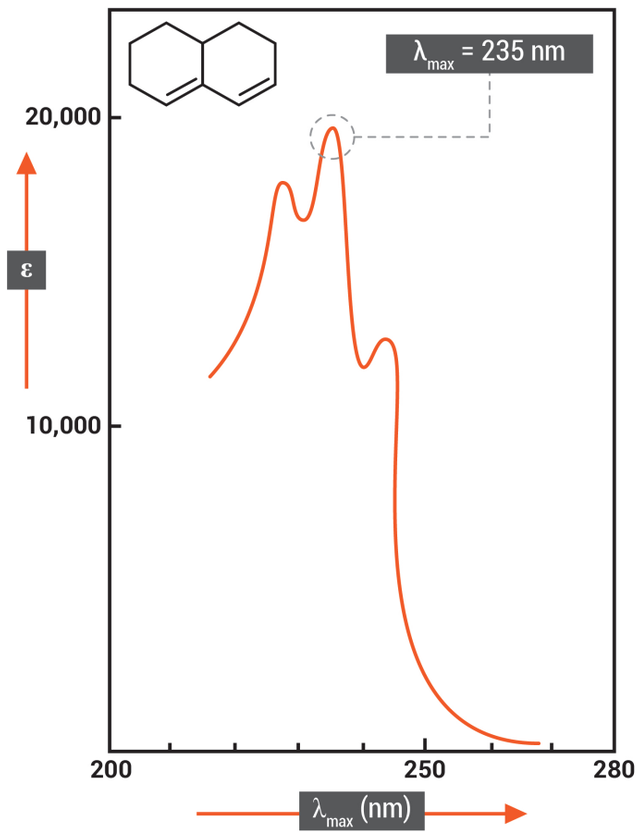

Figure 3.12. *The Absorption Spectrum of a Bicyclic Diene*
(Source: https://www2.chemistry.msu.edu/faculty/reusch/virttxtjml/spectrpy/uv-vis/spectrum.htm)

THE ABSORPTION SPECTRUM - QUANTITATIVE ANALYSIS

1. Prepare the samples as described in the protocols in the monographs. Fill several cuvettes with varying concentrations of a known standard. Clean the sides of the cuvette and read the absorbance of the standards. A typical standard curve will have at least 5 concentrations of the reference standard, done in triplicates.

2. Within the time duration at which the color observed is stable, the samples are read using the spectrometer.

3. After the absorbance readings of standards are obtained, the samples are now added to cuvettes and absorbance measurements are taken.

4. Two methods are employed in determining the concentration of the sample using the absorbance readings.

    a. Standard addition method. If the concentrations of samples are very low or falls below the LOQ, or if matrix effects are expected, the standard addition method is employed. Standard addition analysis is done by measuring the absorbance of samples "spiked" with known and different concentrations of a known standard. The absorbance is plotted (y-axis) vs the amount of standard or known analyte added (x-axis). Regression line is calculated starting from the point on the x-axis at which absorbance or y = 0.

    b. Standard calibration method. This is the usual method of choice for determining the concentration of the analyte by comparing its absorbance readings with the absorbance of a set of standard samples of known concentration. The method is as follows:

        i. Prepare standard solutions containing varying concentrations of pure standard or analyte.

        ii. Read the absorbance at the wavelength in which the analyte absorbs maximally and as described in the monographs.

        iii. Plot the absorbance reading (y-axis) vs the concentration of the analyte (x-axis).

DATA ANALYSIS: THE STANDARD CURVE

A plot of the absorbance of varying concentrations of a known reference standard is the standard curve. ***Figure 3.13*** is a typical calibration curve for determining quantity of absorbing compound (analyte) in solution.

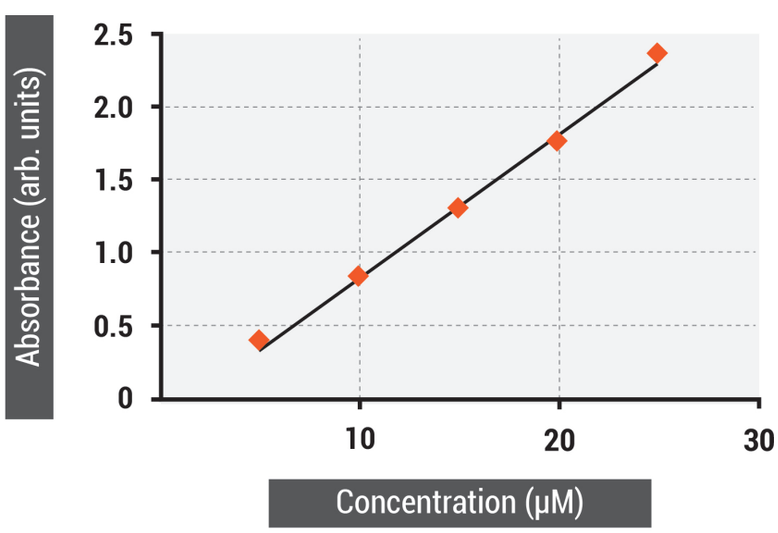

Figure 3.13. *A Typical Calibration Curve*

The slope of the graph of the absorbance vs concentration of the analyte will indicate the sensitivity of the analysis. When the linear least squares regression equation is used, the slope of the best fit line will indicate sensitivity of the measurement. This is denoted by R^2; the closer R^2 to 1.0, the more sensitive the measurement is. Thus, a steeper plot indicates higher sensitivity which is the ability to differentiate small differences in the sample concentration.

SYSTEM REPEATABILITY

To ensure reliability of the UV-Vis data, absorbance readings should be repeated, i.e. technical replicates must be done. In general, a minimum of three replicate trials will ensure reliability of the readings. Replicates can be increased if the readings have high standard deviations (SD) or errors (SE). The calculated concentration of the unknown sample is usually reported as the average of the replicates plus/minus the standard deviation.

APPLICATIONS

Majority of analytical procedures using UV-Vis spectroscopy is conducted to determine concentrations of analytes rather than identify them. It is the common technique employed for chemical analysis because most molecules will absorb at the UV and visible wavelength range. In addition, UV-Vis spectroscopy is also employed in the quantitation of bacterial cultures, purity and determination of the quantity of nucleic acids, quality control in the beverage industry, drug analysis, etc.

FLUORESCENCE SPECTROSCOPY

Fluorometry is a method to measure the intensity of fluorescence or phosphorescence emitted from a solution of fluorescent substance irradiated with an exciting light in a certain wavelength range. Like other spectrophotometric methods, the intensity of fluorescence emission of the analyte is proportional to the concentration of the compound being analyzed. The concentration is usually given in moles/liter of the solution.

PRINCIPLE AND METHODS

All forms of light emission is called luminescence. Fluorescence is a type of luminescence caused when photons of light, usually in the ultraviolet range, are absorbed by chemical compounds exciting it from its singlet ground state to a singlet excited state. When the compound returns to its ground lower energy state, the energy previously absorbed is released in smaller quanta called fluorescence. Below is the Jablonski diagram showing differences in the transitions between a molecule's energy states and light emissions generated.

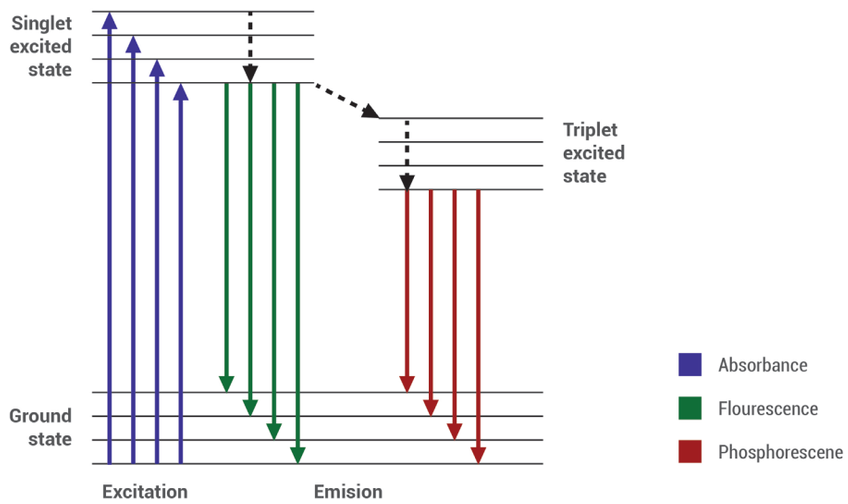

Figure 3.14. *Jablonski Diagram Showing the Transitions in Energy States of Molecules*
(Source: https://jascoinc.com/learning-center/theory/spectroscopy/fluorescence-spectroscopy/)

The emitted photons observed as intensity are detected as a function of wavelength. Fluorescence spectra are observed at higher wavelengths in the UV region. The intensity is measured relative to a standard solution.

There are two spectral types: emission and excitation. The excitation spectrum is obtained by plotting intensities of fluorescence of a sample in solution at varying excitation wavelength but at a fixed emission wavelength. Conversely, the emission spectrum is obtained when the excitation wavelength is fixed while the emission wavelength is varied. Emission spectrum occurs at higher wavelengths than the excitation or absorbance spectrum. The shape of the two spectra are mirror images of each other and are used to see how the sample changes under conditions of varying temperatures, concentration or when the fluorescent compound interacts with other molecules (e.g. quenchers) in the solution.

Molecules that give off fluorescence usually have aromatic rings or pi-conjugated electrons in them. It should also be noted that fluorescence intensity is affected by concentration, temperature, pH of solution and the nature and purity of the solvents used for the sample.

EQUIPMENT

A spectrofluorometer is used and consists of the following:

1. **A light source.** There are different light sources which are chosen based on the application. These include incandescent xenon arcs, high-pressure mercury-vapor lamps, Xe-Hg arc lamps, Hg-Ar lamps, quartz-tungsten halogen (QTH), LED light sources, etc.

2. **An excitation and emission monochromator** to be able to measure both excitation and emission spectra. Monochromators are either prisms or diffraction gratings. These provide the chromatic lights with a wavelength range of 10 nm.

3. **Optical filters.** There are two types of filters: 1) a colored filter which are categorized as monochromatic filter and long-pass filter and 2) a thin film filter. Filters may also be glass filters or solutions in cuvettes. Filters are used to further purify the light coming from the light source and isolate one particular wavelength from a source emitting a line spectrum.

4. **Detector.** The standard detector used is either a general purpose back-illuminated indium-gallium-arsenide (InGaAs) photodiodes or InGaAs photodiode arrays.

5. **Read-out devices.** This may be a meter or a digital display that can send its outputs directly to a computer with a printer system.

6. **Sample holders.** Cuvettes may either be circular, square or rectangular and made of material that will transmit both incident and emitted light.

Below is a typical schematic design of a fluorometer.

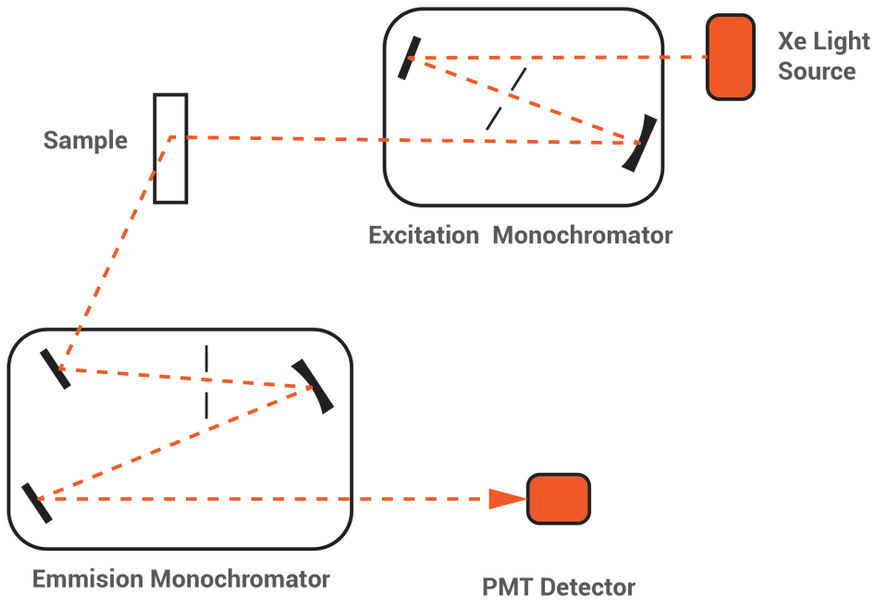

Figure 3.15. *A Typical Schematic Design of a Fluorometer*
(Source: https://jascoinc.com/learning-center/theory/spectroscopy/fluorescence-spectroscopy/)

GENERAL PROCEDURE

1. Analytes are prepared in accordance with appropriate protocols and as described in the monographs.

2. All glasswares should be acid-washed or cleaned using inorganic cleansing agents. Care must be taken that no contaminants such as traces of phenol from the use of filter papers, stopcock grease which contains organic fluorescent compounds, and microbial contamination are present in the solvent or solutions used for the analyte.

3. If water is the solvent for the analyte, the use of deionized-distilled water is recommended. In addition, solvents to be used for fluorescent spectroscopy as well as other reagents must be of high quality and stored in glass containers; plastics can leach organic additives and plasticizers.

4. A blank reading must be taken prior to analysis of the sample. If the blank reading is too high, distilling the solvents used, or repeat washing of containers may reduce this. It is also recommended to determine the absorbance of the solution containing the analyte before the fluorescence analysis and the concentration adjusted to provide a solution with an absorbance of <0.1 A.

APPLICATIONS

An advantage of spectrofluorometry is its high sensitivity (~ 10-100 times more than UV-vis spectroscopy) and the very low quantity of sample requirement. It is therefore a useful tool for trace analysis. It is also able to measure transient changes in the photophysical processes of molecules over time, as well as interactions with its microenvironment. Some applications include quantification of individual components of a mixture in solution, including natural products, analysis of contaminants and classifying substances such as differentiating between aged and unaged wines.

ATOMIC ABSORPTION SPECTROSCOPY

PRINCIPLE

Atomic absorption spectroscopy is useful for analytes that cannot be quantified using color indicators or UV/Flourescence absorption. The two principal forms of atomic spectroscopy are based on absorption and emission.

► Atomic absorption spectroscopy

Atomic absorption (AA) spectroscopy measures the absorption of optical radiation by analyte atoms in its gaseous state which is proportional to its concentration. The most common type is flame AA (FAAS). Other forms are graphite furnace (GFAAS) and cold vapor or hydride atomic absorption spectroscopy.

The method uses the principle that the concentration of an element in the sample being analyzed can be determined by measuring the absorption of the ground state element as light of specific wavelength passes through an atomic vapor layer of the element being measured. It is used to detect metal contaminants, particularly heavy metals. In this method, the atom is subjected to electromagnetic radiation at a specific wavelength to raise the ground state to an excited state. Energy is absorbed at the resonance frequency causing the atom to absorb energy. The energy absorbed is proportional to the concentration of the element present. The excited state is achieved by either atomization by a) flame, b) electrothermal vaporization in a graphite furnace, c) cold vapor or hydride technique, in the case of mercury or certain hydride-forming elements. The detection limit of Flame AAS (FAAS) is 1–100 µg L–1. Electrothermal vaporization is used to determine the concentration of metals that are present in trace quantities such as with many biological samples and environmental unpolluted samples such as fresh water. The limit of concentration detectable for the electrothermal vaporization or graphite furnace AAS (GFAAS) is a factor of 20-200 lower than for FAAS (https://onlinelibrary.wiley.com/doi/abs/10.1002/9780470027318.a0805) or a sensitivity 100-1000 times better than FAAS (https://www.perkinelmer.com Cold vapor (CVAAS) or hydride technique is used to detect mercury, or certain hydride-forming elements such as arsenic, antimony, bismuth, selenium and tin.

► Optical emission spectroscopy

Optical emission spectroscopy is employed for simultaneous determination of several elements at a time. There are two (2) forms-flame OE and inductively-coupled plasma (ICP) OE. Inductively-coupled plasma can either be Inductively-coupled plasma optical emission spectroscopy (ICP-OES) or inductively-coupled plasma mass spectroscopy. ICP-OE has enabled the measurement of larger number of elements at the same time (up to 73 elements) since it measures light emitted from elements in an argon plasma which reaches temperatures of 10,000 K. This high temperature completely atomizes the sample elements and minimizes chemical interference.

The determination of metal contaminants using atomic absorption spectrometry will be outsourced (Refer to the FDA list of accredited analytical laboratories).

EQUIPMENT

For AS, the equipment consists of the following:

1. **A radiation or primary light source.** The light source is usually a hollow cathode lamp (HCL)
2. A sample introduction and nebulization system
3. **An atomizer.** There are three types of sample atomizers:
    a. Flame for FAAS and flame OE. The burner in FAAS is supplied by either air-acetylene or nitrous oxide-acetylene which will generate the flame.

b. Electrothermal graphite tube or furnace for GFAAS,

c. Cold vapor and hydride technique. Cold vapor is most useful for mercury and for certain hydride-forming elements such as arsenic, antimony, bismuth, selenium and tin.

4. **A monochromator to** isolate the specific wavelength of light to be measured

5. **A detector.** The optical detector is a photomultiplier tube (PMT)

6. **A data-acquisition unit** that consists of a computerized instrument control, data collection and analysis. A typical schematic drawing of an AAS is shown below (***Figure 3.16***)

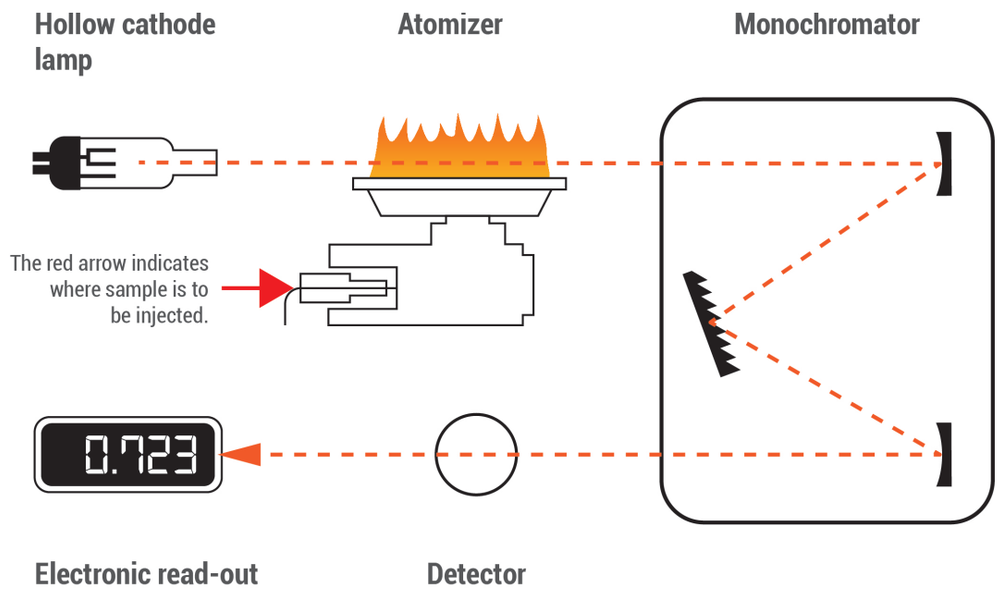

Figure 3.16. *Schematic Drawing of the Components of an AAS*
(source: https://www.agilent.com/en/support/atomic-spectroscopy/atomic-absorption/flame-atomic-absorption-instruments/how-does-aas-work-aas-faqs)

GENERAL PROCEDURE

1. Optimize conditions for the determination, i.e. use matrix modifiers, releasing agents or high temperature to minimize interferences.

2. Correct for background absorption by using blank solutions which consist of the solvent or solution used without the analyte.

3. Select the suitable wavelength and slit width for the specific element.

4. Check details of the temperature program, preheating, drying, pyrolysis, atomization with ramp and hold times. Check inert gas flow.

5. Prepare samples by dissolving in appropriate solvent as described in the monograph, or by digestion, ignition, or a combination to clear up the sample matrix or remove other contaminants. Typical sample preparations involve digestion with concentrated acid such as HNO_3, HCl or H_2SO_4. After digestion, samples are diluted as needed, to

minimize physical interferences. Then these can directly be injected into flame AAS (FAAS) or graphite furnace AAS (GFAAS).

6. Unless otherwise stated in the monograph, ignition temperatures should not exceed 600°C, which could volatilize some metals.

7. Use a blank solution (solvent used without the sample) to adjust the instrument reading to indicate maximum transmission.

8. Read the absorbance of the samples (do replicates), rinsing in between readings.

9. Rinse with water or acidified water after completing the analysis.

DATA ANALYSIS

As with UV-Vis spectrometry, determination of the concentration of the analyte in the unknown sample can be done two ways:

1. **Standard addition.** Solutions can be prepared containing the test solution. Add increasing concentrations of a reference standard to the test solutions, except for one which is the baseline concentration. Read the absorbance of the test solutions. Use the same number of replicates for all test solutions. A plot of the absorbance vs the concentration of the reference standard added is made. Regression line is calculated starting from the point on the x-axis at which absorbance or y is equal to zero.

2. **Calibration method.** A calibration curve can be constructed by plotting the absorbance readings obtained for reference standard solutions vs the concentration of these solutions. The concentration of the analyte in the unknown sample can be determined from the standard curve.

APPLICATION

Atomic absorption spectroscopy is employed in a number of industries and researches. These include:

1. Agriculture industries and research

2. Environmental science research

3. Forensics

In agriculture and environmental science, it is used to determine the presence of heavy metals or potentially harmful elements in soils and in the environment. It is also used to detect metal poisoning such as mercury, arsenic, lead, etc. Forensic scientists can also use the method to analyze blood and other tissue samples for gunshot powder residues.

<42> CHROMATOGRAPHIC TECHNIQUES

Chromatography is a separation technique that consists of multi-stage separation wherein the components of a mixture are distributed between two phases: a stationary and a mobile phase. The stationary phase may be solid, liquid supported on a solid (e.g. TLC plates) or a gel. It may also be packed in a column, spread as a layer, or distributed as a film. The mobile phase may be gaseous (gas chromatography), or liquid. The separation is based on the affinity or adsorption of the component to the matrix (adsorption chromatography) or its solubility in phases of different polarities (partition chromatography). It may also be based on differences in the physicochemical properties of the molecules being separated, such as size, charge, mass, binding affinities, etc. Chromatography works on the principle that different compounds will have different solubilities and adsorption to two phases between which they are to be partitioned. (European Pharmacopeia)

For analytical purposes, i.e. identification of components of a sample, the monographs herein included utilize a partition type chromatography on a solid support—a thin layer of matrix on glass or aluminum plates.

TLC /HPTLC

PRINCIPLES

Thin-layer Chromatography is a method to separate components of a mixture based on their solubility in different phases of a solvent system consisting of a mixture of mobile and stationary phases. The stationary phase interacts with usually a thin-layer matrix; compounds soluble in the stationary phase will move slower than the solvent front. Solvents that do not bind or interact with the thin-layer matrix will move faster carrying with it the compounds which dissolve in the solvent. High performance thin-layer chromatography uses the same principle. It is an enhanced modification of TLC using automation of the different steps for better resolution and quantification. TLC/HPTLC is applied for the resolution and identification of constituents of a mixture, and the determination of the purity of these substances. (see: Japanese pharmacopeia)

EQUIPMENT

The equipment consists of the following:

- Plates: aluminum plates coated with uniform 200 m layer of porous (60-Å pore size) silica gel and irregular particles of 2-10 m and average particle size of 5 m, a polymeric binder, and a fluorescence indicator (e.g. F254) of 20 x 10 cm.
- For HPTLC, an automatic TLC Sampler for the application of specified volumes of samples as bands with specified length at specified positions. For TLC, this can be done manually.
- For HPTLC, an automatic developing chamber (ADC) with 20 x 10 cm twin trough chamber for controlling the saturation, development and drying. For TLC, a developing chamber made of glass which is equilibrated with solvent before the TLC plates are placed.
- Humidity control module containing MgCl2-6H2O for controlling the activity of the stationary phase via relative humidity (33% at 20°C).
- TLC sprayer for treatment of the plate with derivatization reagent, if necessary.
- TLC Visualizer for documentation of chromatograms under UV 254 nm, UV 366 nm, and white light. (computer programs to quantitate – Quantiscan)

THIN-LAYER CHROMATOGRAPHY

The apparatus used in thin-layer chromatography consists of the TLC plates that contain the stationary phase, a developing

chamber (TLC chamber) and the mobile phase consisting of at least a binary (2-component) solvent system. Below is the separation of mixtures of compounds in the TLC chamber.

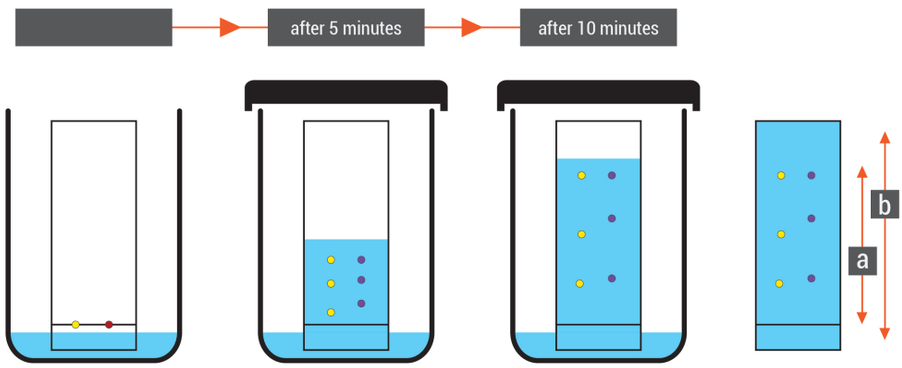

Figure 3.17. *Steps of Thin-Layer Chromatography*

HIGH-PERFORMANCE THIN-LAYER CHROMATOGRAPHY

The apparatus used for HPTLC is similar to that used for TLC except that the process is fully automated from automatic sampling, developing, scanning and documentation.

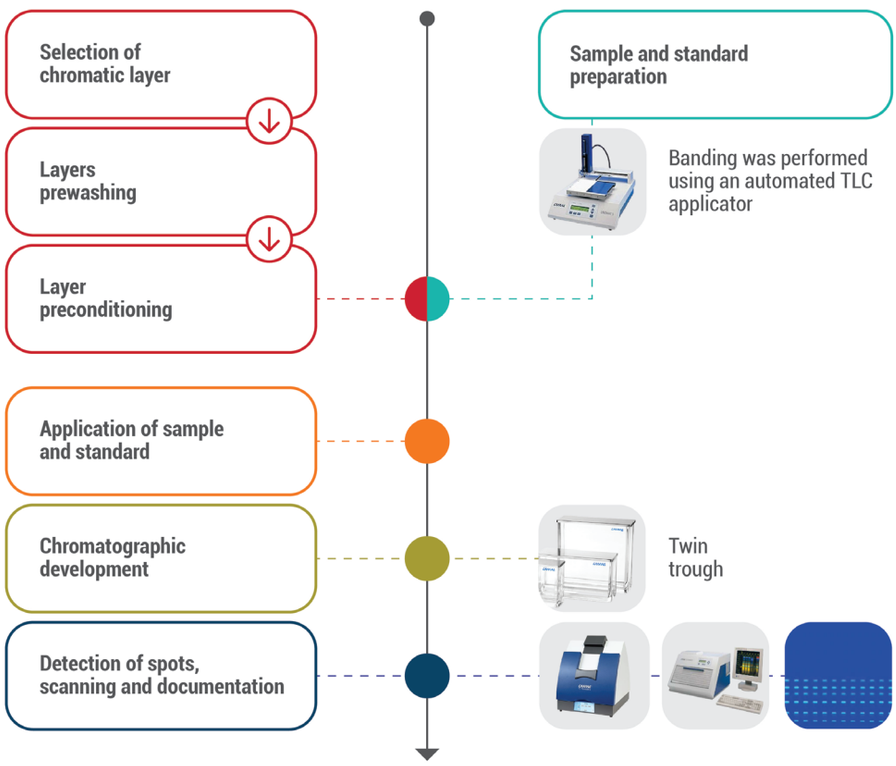

Figure 3.18. *Schematics of the HPTLC Workflow*
(Source: https://www.researchgate.net/publication/353222117_High-Performance_Thin_Layer_Chromatography_In_The_Pharma_Industry)

PROCEDURE

► Preparation of Test Solution

Dry extract or fraction of the plant sample (quantity to be optimized) is prepared as described in the monographs. This is dissolved in 1 mL methanol or ethanol, and then sonicated for 15 min. The test solution is filtered using a 0.45 mm Nylon syringe filter and the filtrate is used as the sample solution.

► Sample Application and Plate Layout

Samples are applied as narrow bands placed 8.0 +/- 0.5 mm from the lower edge of the plate. Standards are also applied on the lane nearest to the edge, no less than 20 mm from the edge of the plate. Distance between tracks (center to center) should not be less than 11.0 +/- 0.5 mm. Application volumes are specified in each monograph 2.0-10.0 uL. Solvent front is electronically set and detected by electronic developing chamber and visualizer, respectively, for HPTLC or visually determined in TLC.

► Preconditioning

After sample application, the plates are preconditioned at relative humidity of 33% in the developing chamber for at least 10 min.

► Developing chamber and plate development

For HPTLC: The rear trough of the twin trough chamber is fitted with a saturation pad (filter paper). The chamber is filled with 10 mL of developing solvent and 25 mL preconditioning solvent (same solvent as developing) to wet the saturation pad and achieve 5 mm developing solvent level on both troughs. Saturation of the closed chamber is 20 min, and then the plate is introduced in horizontal position into the front trough with the coated layer facing the saturation pad. When the set solvent front is reached, ADC2 will automatically remove the plate from the chamber for drying.

For TLC: For single-chamber developer, the chamber is fitted with a saturation pad or filter paper. The chamber is then filled with developing solvent to wet the saturation pad and achieve 5 mm developing level. The chamber is covered (with glass plate) to allow for saturation of the closed chamber. This will be for about 20 min. Then the plate is introduced in the chamber (horizontal or vertical, depending on the size of the chamber), with the coated layer facing the saturation front. The solvent will be allowed to diffuse through the plate, until a few mm from the top of the plate.

► Derivatization

If derivatization is needed, 1-2 mL of derivatizing reagent is homogeneously sprayed onto the plate.

► Visualization

The plate chromatograms is visualized under UV 254 nm, UV 366 nm, and white light prior to and after derivatization.

► System Suitability and Reference Standards

Reference standards are prepared in the same manner as the test samples or analytes.

► Evaluation and Acceptance Criteria*

The use of a reference standard will enable comparison of the sample and standard solutions based on the set *Acceptance Criteria* section of the monograph with respect to zone position, zone separation, color and relative intensity.

DATA ANALYSIS

Retention factor, also called retardation factor, Rf, is the ratio of the distance from the point of application to the center of the spot or band (a) and the distance travelled by the solvent front from the point of application (b) as shown in the figure.

$$Rf = \frac{\textit{distance travelled by the component (a)}}{\textit{distance travelled by the solvent (b)}}$$

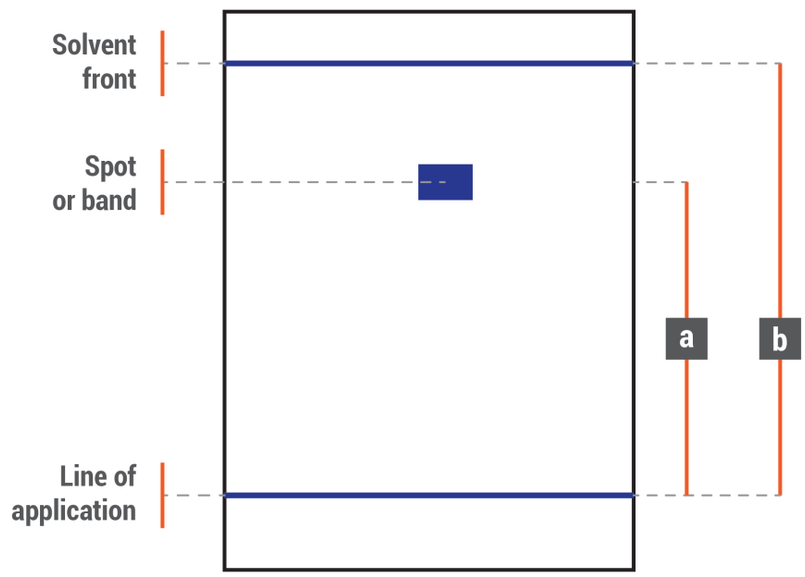

Figure 3.19. *A Typical Chromatogram*

APPLICATION

Thin-layer chromatography and its advanced automated HPTLC is used in various industries and analysis. It can be used to analyze and detect many substance classes, including pesticides and phytochemicals such as steroids, alkaloids, lipids, sugars, and glycosides, phenolics, and other biochemicals such as nucleotides. It has been used to detect and quantify drugs, caffeine and other natural products from various sources.

V. Measurements, Tests and Assays

<51> IDENTIFICATION TESTS AND ASSAYS

Identification tests are provided for the verification of the identity of the substance described in the monograph. Such tests, however specific, are not necessarily sufficient to establish proof of identity but failure of an article taken from a labeled container to meet the requirements of a prescribed identification test indicates that the article maybe mislabeled and will require confirmatory test(s). [PP1]

Herbal preparations are crucially identified using microscopical and high performance thin-layer chromatography (HPTLC). [THPP 2020] HPTLC is mainly used to verify the chemical characteristics of the herbal preparations. The HPTLC chromatograms illustrates the separation of the chemical constituents of the preparation which is the chemical fingerprints of the preparation extract. In the monograph, the concentration of each standard solution of known constituent marker is compared with the herbal preparations.

Any separation obtained is based upon the principles of adsorption, partition, or a combination of both effects, depending on the particular type of support, its preparation, and its use with different solvents. [PP1]

TESTS AND ASSAYS

The tests and assays described are official methods upon which the standards of the Philippine Pharmacopeia depend. The analysts may employ alternative methods, including methods of microanalysis, in any tests and assays if they have proven that the method that they use will give the result of equivalent accuracy.

The tests and assays for herbal medicines are based on Administrative Order No. 172 series of 2004.

The test for synthetic substances as per claimed indications such as Aspirin, Paracetamol, Dipyrone, Phenylbutazone, Pyrazolone, Corticosteroids, Anabolic steroids, Gonadal Hormones, Ephedrine, Sildenafil and related substances, Indomethacin, Chlorphenamine, Aminopyrine, Caffeine and other synthetic drugs that maybe present or added.

<52> MICROBIAL TESTS

MICROBIAL ENUMERATION TEST

https://online.uspnf.com/uspnf/document/1_GUID-0392F79D-1F8A-4B8D-BEC8-C6FD7B39966F_1-en-US.

The tests herein provide an estimation of the number of viable aerobic microorganisms (mesophilic bacteria and fungi) present in the test sample and shows freedom from designated microbial species in pharmaceutical articles of all kinds, from raw materials to the finished form. This test does not include products containing viable microorganisms as active ingredients. Alternative procedures may be used provided they are equivalent to the methods described herein or in individual monographs.

GENERAL PROCEDURES

Observe aseptic precautions in handling the specimens at all stages of preparing for and applying the tests. The term" incubate" means that the container is held in air that is thermostatically controlled at a temperature between 30°C and 35°C for a period of 24 to 48 hours. The term "growth" is used to designate the presence and presumed proliferation of

viable microorganisms. Prior to the conduct of tests, assure results are valid by demonstrating that the test specimen to which they are applied do not inhibit multiplication of microorganisms at prescribed test conditions. The general method uses the membrane filtration or one of the plate-count methods. Inoculation of diluted specimens of the material to be tested is conducted with separate viable cultures of *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Salmonella. [PP1]*

TESTS FOR SPECIFIED MICROORGANISMS

The methods are similar to the microbial enumeration test. Test organisms are prepared as standardized stable suspensions. Seed-lot cultures are maintained to not more than five passages from the original master seed-lot. Bacterial strains are grown in Soybean-Casein digest broth or agar at 30-37°C for 18-24 hours. Candida albicans are grown separately on Sabouraud Dextrose agar or broth at 20-25°C for 2-3 days.

MICROBIAL LIMITS

Table 3.10. For Untreated Plant Material Harvested under Acceptable Hygienic Conditions Intended for Further Processing

ORGANISM	UNIT	LIMIT
Escherichia coli	cfu/g	Negative
Moulds propagules	cfu/g	Maximum 10^5
Staphylococcus	cfu/g	Negative

Table 3.11. Plant Materials that will Undergo Pre-Treatment (E.G. with Boiling Water as Used for Herbal Teas and Infusions) or If the Material is to be Used for Topical Dosage Form

ORGANISM	UNIT	LIMIT
Aerobic bacteria	cfu/g	Maximum 10^7
Yeasts and molds	cfu/g	Maximum 10^4
Escherichia coli	cfu/g	Negative
Salmonellae	cfu/g	Negative
Staphylococcus	cfu/g	Negative
Other Enterobacteria	cfu/g	Maximum 10^4

Table 3.12. Plant Materials to be Used in Drugs for Internal Use

ORGANISM	UNIT	LIMIT
Aerobic bacteria	cfu/g	Maximum 10^5
Yeasts and molds	cfu/g	Maximum 10^3
Escherichia coli	cfu/g	Negative
Salmonellae	cfu/g	Negative
Staphylococcus	cfu/g	Negative
Other Enterobacteria	cfu/g	Maximum 10^3

<53> PHYSICOCHEMICAL TESTS

In addition to microscopic and chromatographic identifications, the quality control tests are to be conducted on herbal preparations. Some of physicochemical specifications include water-soluble extractive, ethanol-soluble extractives, total ash, acid-insoluble ash, water-soluble ash and water contents. [THPP 2020] These are quantitative measurements with criteria and limits specified in individual monographs. Other requirements that may be included to determine quality are volatile oil, tannin contents and foreign matter. The general quality control test for herbal preparation must include macroscopical, microscopical, chemical tests, chromatography and physicochemical tests.

The following are among the physicochemical tests to be conducted as stated in AO No. 172 series of 2004:

- **Tablet:** weight variation, content uniformity, disintegration test, hardness test, friability and microbial test
- **Capsule:** weight variation, content uniformity, microbial test
- **Syrup/Liquid:** viscosity, pH, microbial test
- **Suspension:** suspendability, homogeneity, viscosity, minimum fill, pH, microbial test
- **Ointment/cream/semi-solid preparation:** palpability, homogeneity, pH, melting point, allergenicity and primary irritation test, microbial test
- **Suppository:** allergenicity and primary irritation test, microbial test

<54> MOISTURE CONTENT

Many pharmacopeial articles are either hydrates or contain water in adsorbed form. As a result, the determination of the water content is important in demonstrating compliance with the pharmacopeial standards.

When the article contains water of hydration, *Method I (Titrimetric), Method II (Azeotropic), or Method III (Gravimetric)* is employed, as directed in the individual monograph, and the requirement is given under the heading *Water*. The heading *Loss on Drying* (see Loss on Drying 731) is used in those cases where the loss sustained on heating may not entirely be water. [USP 43/NF38]

Loss on Drying is a method to measure the loss in mass of the sample when dried under conditions specified in each monograph. [PP1]

The titrimetric determination of water is based upon the quantitative reaction of water with an anhydrous solution of sulfur dioxide and iodine in the presence of a buffer that reacts with hydrogen ions.

In the original titrimetric solution, known as Karl Fischer *Reagent*, the sulfur dioxide and iodine are dissolved in pyridine and methanol. The test specimen may be titrated with the reagent directly, or the analysis may be carried out by a residual titration procedure. The stoichiometry of the reaction is not exact, and the reproducibility of a determination depends upon such factors as the relative concentrations of the reagent ingredients, the nature of the inert solvent used to dissolve the test specimen, and the technique used in the particular determination. Therefore, an empirically standardized technique is used in order to achieve the desired accuracy. Precision in the method is governed largely by the extent to which atmospheric moisture is excluded from the system. The titration of water is usually carried out with the use of anhydrous methanol as the solvent for the test specimen. In some cases, other suitable solvents may be used for special or unusual test specimens. In these cases, the addition of at least 20% of methanol or other primary alcohol is recommended. [USP 43/NF38]

► Water Activity

The measurement of water activity or equilibrium relative humidity (ERH) is a key parameter in the quality control of

moisture-sensitive products or materials. While moisture content refers to the total amount of water in a sample, water activity refers to the "excess" water that will react with microorganisms or that will promote microbial growth. Thus, the higher the water activity, the faster microorganisms like bacteria, yeast, and mold will be able to grow which will have an impact on storage requirements and shelf life of a product.

Water activity is generally used to determine stability and shelf life of food products. However, it can be used for other products such as herbal preparations which use dried plant parts or crude preparations. Water activity values may be obtained by either resistive electrolytic hygrometer, a capacitance hygrometer, or a dew point hygrometer. These are also known as water activity meters. The water activity (Aw) of a sample is the ratio between the vapor pressure of the sample, and the vapor pressure of distilled water under identical conditions. A water activity of 0.80 means the vapor pressure is 80 percent of that of pure water. Water activity increases with temperature.

The equipment used to measure relative humidity, with proper modifications, can be used for measuring water activity provided that the sensing element has to be kept away from the environment and in contact only with the sample whose Aw is being measured. This would require a sealed container with the sensor mounted above the test sample.

https://www.fda.gov/inspections-compliance-enforcement-and-criminal-investigations/inspection-technical-guides/water-activity-aw-foods

FOREIGN SUBSTANCES AND IMPURITIES

► <55> Limits for Toxic Metals

Toxic metals limit test is a limit test of the quantity of heavy metals contained as impurities in herbal medicines.

This test is provided to demonstrate that the content of metallic impurities that are colored by sulfide ion, under the specified test conditions, does not exceed the toxic metals limit specified in the individual monograph in percentage (by weight) of lead in the test substance, as determined by concomitant visual comparison (see *Visual Comparison* in the section *Procedure* under *Spectrophotometry and Light-Scattering* (851)) with a control prepared from a *Standard Lead Solution*. [Note—Substances that typically will respond to this test are lead, mercury, bismuth, arsenic, antimony, tin, cadmium, silver, copper, and molybdenum.]

Determine the amount of toxic metals by *Method I*, unless otherwise specified in the individual monograph. *Method I* is used for substances that yield clear, colorless preparations under the specified test conditions. *Method II* is used for substances that do not yield clear, colorless preparations under the test conditions specified for *Method I*, or for substances that, by virtue of their complex nature, interfere with the precipitation of metals by sulfide ion, or for fixed and volatile oils. *Method III*, a wet-digestion method, is used only in those cases where neither *Method I* nor *Method II* can be used. [USP 43/NF38]

For dried plants, toxic metal limits are as follows; Lead 10 ug/g(ppm), Cadmium 0.3 ug/g (ppm), Mercury 0.5 ug/g (ppm) and Arsenic 0.3 ug/g(ppm) as per Administrative Order No. 172 series of 2004.

► <56> Percent Extractives

Alcohol-soluble extractives may be extracted by either hot extraction (Method 1) or cold extraction (Method 2) methods. Similarly water-soluble extractives may also be extracted by either of two methods, following similar procedures except that water is used in place of alcohol. https://online.uspnf.com/uspnf/document/1_GUID-0392F79D-1F8A-4B8D-BEC8-C6FD7B39966F_1-en-US

► <57> Limits for Pesticide Residues

Pesticide residue should conform to the limits as defined by WHO/FAO Codex Alimentarius.

Pesticide applies to any substance or mixture of substance intended to prevent, destroy or control any unwanted species of plants and animals causing harm during or otherwise interfering with the production, processing, storage, transport or marketing of pure articles. These include substances intended for use as growth regulators, defoliants, desiccants and any substance applied to crops before or after harvest to protect the product from deterioration during storage and transport.

Botanical extracts, tinctures, or other pharmaceutical forms might contain pesticide residues at either enriched or reduced levels compared to their native form as plant materials. Unless otherwise indicated in the monograph, the limits for pesticides in extracts of botanical articles are calculated using the formula below.

$$If\ E \leq 10:\ Limit\ (mg/kg) = L \times E$$

$$If\ E > 10:\ Limit\ (mg/kg) = AM/100B$$

where:

- L = the limit in the original article as listed in Table 4 (see Pesticide Residue Analysis under Articles of Botanical Origin 561), or EPA tolerance or the FDA action level;
- E = the plant to extract ratio (i.e. the ratio between the quantity of botanical article used in the manufacture of the extract and the quantity of the extract obtained);
- A = the acceptable daily intake (ADI), as published by FAO-WHO, in mg/kg of body weight;
- M = body weight, in kg (60 kg); and
- B = the daily dose of the extract, in kg.

[Note—The higher pesticide limits for extracts of botanical ingredients may be justified if the suggested intake or dose of the extract is reduced by a factor which is higher than E.]

A total or partial exemption from the test may be granted when the complete history (nature and quantity of the pesticides used, date of each treatment during cultivation and after harvest) of the treatment of the batch is known and can be checked precisely according to good agricultural and collection practice (GACP). [USP 43/NF38]

► <58> Limits for Aflatoxin

Total aflatoxin should conform with the prescribed specification of 20 ug/kg and Aflatoxin B1 of 10 ug/kg by ELISA method or liquid chromatography.

Aflatoxins are carcinogenic secondary metabolites produced by some fungal strains. They are found in agricultural products which include herbal samples and crude herbal drugs. Regulatory limits are set on aflatoxins in herbal preparations since it may be contaminated with aflatoxin and not justt food samples since ingredients of botanical origin have reports of contamination in foreign countries. [JP 17th ed]

Among the methods of detection of aflatoxin are high performance liquid chromatography (HPLC) with fluorescence detection, liquid chromatography-mass Spectroscopy (LCMS) or use of enzyme-linked immunosorbent assay (ELISA) kits.

Aflatoxins are generally purified from samples by cartridge-type columns such as immunoaffinity column which is useful in purification of aflatoxin from crude drug preparation. However, for some multifunctional columns are more effective.

ELISA kits can detect the presence and absence of aflatoxin from the sample using antigen-antibody interactions. While a kit for the detection of total aflatoxin can be used for a qualitative test, instrumental analysis is required in

order to quantify aflatoxin. To confirm the performance of immunoaffinity column spike and recovery test are necessary.

► Assays

» Markers and Reference Standards (WHO guidelines for selecting marker substances of herbal origin for quality control of herbal medicines. Annex 1)

The challenge in the use of herbal medicines is the variability of constituents in the plant source due to genetic and environmental factors. More often, the nature of the active components may be unknown or diverse and has not been established. Where quality of the raw materials must be ascertained, chemical and chromatographic examination help to provide batch-to-batch comparability and the chromatogram may be used as a "fingerprint" for the herbal ingredient by demonstrating the profile of some common plant constituents. Reference standards may be used to provide confidence about the quality of the raw materials used for the herbal preparation.

Reference standards are chemically defined molecular entities used in standardization or quality control of herbal drugs and herbal materials.

Markers are reference substances that are indicative of the constituents of a herbal material. Markers may or may not be the active principles, i.e. they may or may not contribute to the therapeutic activity, but are used to determine identity.

"Primary chemical reference substances are substances that are widely acknowledged to have the appropriate qualities within a specified context, and whose assigned content when used as a (mostly as an assay) standard is accepted without requiring comparison to another chemical substance" (WHO Annex 1).

"Secondary chemical reference substances (also called working standards) are substances whose characteristics are assigned and/or calibrated by comparison with a primary chemical reference substance" (WHO Annex 1).

» Phytochemistry

Phytochemical screening technique is a qualitative evaluation that identifies and characterizes crude herbal preparations with respect to its phytochemical constituents. Phytochemicals or natural products are small molecules with a variety of functions. These are produced by living organisms in response to environmental pressures. There are various types of phytochemicals including phenolics, terpenes, steroids, indoles, carbohydrates, anthrones and anthraquinones.

Phytochemical analysis employs qualitative and analytical techniques to detect the constituents of plants that may be used for herbal preparations. After botanical identification, the dried plant material is extracted with appropriate solvent and then constituents are separated usually using paper chromatography. Then, various spray reagents are used to confirm the presence of these chemical entities in the herbal preparations.

Equipment

The equipment used is as described in *Thin-Layer Chromatography*. Alternatively, paper chromatography may also be employed.

General Methods

Samples are applied to TLC plates or paper. The components are allowed to separate in a developing chamber and then sprayed with appropriate spray reagent given in the table. Observe and view under visible light and at 365 nm.

Table 3.13. Tests For Phytochemicals Using Spray Reagents and their Positive Results

PHYTOCHEMICALS	SPRAY REAGENT	STANDARD	POSITIVE RESULT
Alkaloids	Dragendorff's reagent	Quinine	Brown-orange spots immediately after spraying viewed under visible light (Note: Colors are unstable)
Flavonoids	Antimony (III) chloride	Quercetin	Intense yellow to orange visible zones under visible light for glycosidic flavonoids Fluorescent colors under 365 nm
Tannins	Potassium ferricyanide-ferric chloride	Tannic Acid	Blue spots visible under visible light
Indoles	Van-Urk Salkowski reagent	Indole	Blue-violet spots under visible light
Triterpenoids	Vanillin-sulfuric acid	Saponin	Triterpenes and sterols: blue-violet spots under visible light Essential oils: wide range of colors under visible light
Anthraquinones	Magnesium acetate	Anthraquinone	Orange-violet color under visible light
Anthrones, coumarines	Borntrager reagent	Anthrone	Anthraquinones: orange zones under visible light Anthrones: yellow zones under UV 365nm Coumarins: blue zones under UV 365nm
Sugars	α-naphthol-sulfuric acid	sugars	Blue spots under visible light

Below in ***Figure 3.20*** are some examples of phytochemical detection of components of samples using the spray reagents listed in the Table.

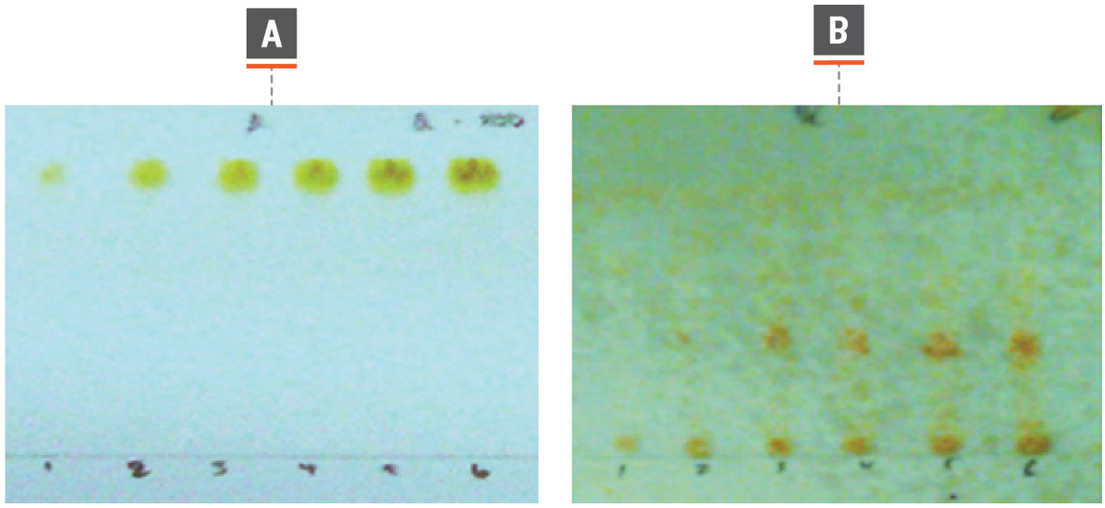

Figure 3.20. *TLC Profile*

TLC profile (***Figure 3.20***) of varying concentrations of the standards for (A) flavonoid and (B) alkaloids. Flavonoids were detected by antimony (III) chloride, while Dragendorff's spray reagent was used for the detection of alkaloids.

» Reagents and Solutions

The reagents, test solutions and volumetric solutions mentioned in this publication are described below. Reagents are denoted by the abbreviation R, test solutions by the abbreviation TS, and volumetric solutions by the abbreviation VS. The concentration of the reagent solutions is expressed in g/L, that is, grams of anhydrous substance per liter of water or solvent, as indicated. Where no solvent is indicated, demineralized water should be used. The procedures for the preparation of test solutions that require special attention are given in detail below. The designation of d denotes the relative density d_{20}, i.e. measured in air at 20 °C in relation to water at 20 °C. Color index (CI) numbers are provided for stains.

Acetic acid, glacial, R. $C_2H_4O_2$; *d* ~1.048.
A suitable commercially available reagent.

Acetic acid (~300 g/l) TS.
A solution of glacial acetic acid R containing about 300 g of $C_2H_4O_2$ per litre (approximately 5 mol/l); *d* ~1.037.

Acetic acid (~60 g/l) TS.
Acetic acid (~300 g/l) TS, diluted to contain about 60 g of $C_2H_4O_2$ per litre (approximately 1 mol/l); *d* ~1.008.

Acetic acid, dilute.
Dilute 6 g of glacial acetic acid with water to make 100 mL (1 mol/l).

Acetone R. C_3H_6O.
A suitable commercially available reagent.

Acetonitrile R. Methyl cyanide, C_2H_3N.
Description. A clear, colorless liquid.
Miscibility. Freely soluble with water.
A suitable commercially available reagent.

Acidic tin (II) chloride TS.
Dissolve 8 g of tin (II) chloride dihydrate in 500 mL of hydrochloric acid. Preserve in glass-stoppered bottles. Use within 3 months.

Aflatoxin mixture TS.
Procedure. Prepare a mixed working standard in a mixture of 98 volumes of chloroform R and 2 volumes of acetonitrile R, containing 0.5 µg of each of aflatoxins B1 and G1 per mL, and 0.1 µg of each of aflatoxins B2 and G2 per mL.
Note. Aflatoxins are highly toxic and should be handled with care. National legal requirements should be followed. Suitable commercially available working standards.

Agar R.
A suitable commercially available reagent.

Alpha-naphthol reagent, 15%
Dissolve 15 g α-naphthol in ethanol (15% α-naphthol) to make up 100 mL as stock solution

Alpha-naphthol-sulfuric acid solution
Mix 10.5mL of the 15% α-naphthol in ethanol with 6.5 mL concentrated sulfuric acid, pouring slowly. In a separate container, mix 40.5mL ethanol and 4mL water. Mix with the α -naphthol-sulfuric acid solution. For phytochemical testing, spray the TLC plates with the reagent, then heat the sprayed TLC plates at 100°C for 3-6 mins.

Aluminium chloride R. $AlCl_3.6H_2O$.
A suitable commercially available reagent.

Aluminium oxide, purified, R. Al_2O_3.
A suitable commercially available reagent for column chromatography.

Amaranth S R. C.I. 16185; acid red 27; $C_{20}H_{11}N_2Na_3O_{10}S_3$.
Description. A deep brown or deep reddish brown, fine powder. A suitable commercially available reagent.

Anisaldehyde R. 4-Methoxybenzaldehyde; $C_8H_8O_2$.
Description. A colorless to pale yellow, oily liquid with an aromatic odor.
Boiling point. About 248°C.
Mass density (ρ_{20}). About 1.125 kg/l.
A suitable commercially available reagent.Anisaldehyde TS.

Anisaldehyde TS.
Procedure. Mix in the following order: 0.5 mL of anisaldehyde R, 10 mL of glacial acetic acid R, 85 mL of methanol R and 5 mL of sulfuric acid (~1760 g/L) TS.

Antimony (III) Chloride solution, 10%
Procedure. Dissolve 10 g antimony (III) chloride in chloroform, adding enough of the solvent to make 100 mL of spray solution.

Argon R. Ar.
Contains not less than 999.95 mL of Ar per liter. A suitable commercially available reagent.

Argon-methane R.
A suitable commercially available reagent.

Arsenic, dilute, AsTS.
One milliliter contains 10 µg of arsenic.
Procedure. Dilute 1 mL of strong arsenic AsTS with sufficient water to produce 100 mL.
Note. Dilute arsenic AsTS must be freshly prepared.

Arsenic, strong, AsTS.
Procedure. Dissolve 0.132 g of arsenic trioxide R in 6 mL of sodium hydroxide (~80 g/L) TS, by gentle heating. Dilute the cooled solution with 20 mL of water, and add 50 mL of hydrochloric acid (~250 g/L) TS and sufficient water to produce 100 mL.

Arsenic trioxide R. As_2O_3.
A suitable commercially available reagent.

Benzoic acid R. $C_7H_6O_2$. Contains not less than 99.8% of $C_7H_6O_2$.
Description. Colorless, light, feathery crystals or a white, microcrystalline powder; characteristic, faint odor.
Solubility. Slightly soluble in water; freely soluble in ethanol (~750 g/l) TS, ether R, and chloroform R.
Methanol-insoluble substances. Dissolve 20 g in 200 mL of methanol R and digest under complete reflux for 30 min. Filter through a tared filtering crucible, wash thoroughly with methanol R, and dry at 105°C; leaves a residue of not more than 1.0 mg.
Assay. Dissolve about 0.5 g, accurately weighed, in 15 mL of ethanol (~750 g/L) TS, previously neutralized to phenol red/ethanol TS, add 20 mL of water and titrate with sodium hydroxide (0.1 mol/L) VS, using phenol red/ethanol TS as indicator. Repeat the operation without the substance being examined and make any necessary correction. Each mL of sodium hydroxide (0.1 mol/l) VS is equivalent to 12.21 mg of $C_7H_6O_2$.

Bile salts R.
Description. A concentrate of beef bile, the principal constituent of which is sodium desoxycholate, determined as cholic acid.
Solubility. Soluble in water and in ethanol (~750 g/L) TS.

Acidity. pH of a 0.02 g/mL solution 5.8–6.2.
A suitable commercially available reagent.

Borntrager reagent (5% potassium hydroxide in methanol)
Dissolve 5 g potassium hydroxide in methanol. Add enough methanol to make 100 mL solution.

Brilliant black BN R.
C.I. 28440; $C_{28}H_{17}N_5Na_4O_{14}S_4$.
Description. A bluish violet or greyish black powder or fine crystals.
Solubility. Freely soluble in water, practically insoluble in ethanol (~750 g/l) TS, acetone R, chloroform R and ether R.
A suitable commercially available reagent.

Brilliant green R.
Malachite green G; basic green 1; C.I. 42040; $C_{27}H_{34}N_2O_4S$.
Description. Small, glistening golden crystals.
Solubility. Soluble in water and ethanol (~750 g/l) TS.
A suitable commercially available reagent.

Bromine R. Br2.
A suitable commercially available reagent.

Bromine AsTS.
Procedure. Dissolve 30 g of potassium bromide R in 40 mL of water, add 30 g of bromine R and dilute with sufficient water to produce 100 mL. The solution complies with the following test. Evaporate 10 mL nearly to dryness on a water- bath, add 50 mL of water, 10 mL of hydrochloric acid (~250 g/l) AsTS, and sufficient stannous chloride AsTS to reduce the remaining bromine, and apply the general test for arsenic. The color of the stain produced is not more intense than that produced from a 1-mL standard stain, showing that the amount of arsenic does not exceed 1 µg/mL.

Bromocresol purple R.
$C_{21}H_{16}Br_2O_5S$.
A suitable commercially available reagent.

Calcium carbonate R1.
$CaCO_3$.
A suitable commercially available reagent.

Calcium carbonate R2.
Calcium carbonate R1 of suitable quality to serve as a primary standard for the standardization of disodium edetate solutions.
A suitable commercially available reagent.

Calcium hydroxide, saturated at 25°
Add $Ca(OH)_2$ to water until saturation is exceeded at 25°. Use water that has been recently boiled and protected from the atmosphere to limit carbon dioxide absorption. Then filter or decant.

Calcon R.
Monosodium salt of 2-hydroxy-1-[(2-hydroxy-1-naphthyl)azo] naphthalene-4-sulfonic acid; C.I. Mordant Black 17, C.I. 15705, Eriochrome Blue Black R, Solochrome Dark Blue; $C_{20}H_{13}NaO_5S$.
A suitable commercially available reagent.

Calcon carboxylic acid R.
2-Hydroxy-1-(2-hydroxy-4-sulfo-1-naphthylazo)-3- naphthoic acid; $C_{21}H_{14}N_2O_7S,3H_2O$.
Description. A dark-brown powder with a violet tint.
Solubility. Practically insoluble in water; slightly soluble in methanol R and in ethanol (~750 g/L) TS; freely soluble in solutions of alkali hydroxides.
A suitable commercially available reagent.

Calcon carboxylic acid indicator mixture R.
Procedure. Mix 0.1 g of calcon carboxylic acid R with 10 g of anhydrous sodium sulfate R.

Calcon indicator mixture R.
Procedure. Mix 0.1 g of calcon R with 10g of anhydrous sodium sulfate R.

Carbophenothion. $C_{11}H_{16}ClO_2PS_3$.
O,O-Diethyl S-[[(4-chlorophenyl)thio] methyl]- phosphorodithionate.
Yellowish liquid, practically insoluble in water, miscible with organic solvents. d4, about 1.27.
A suitable commercially available reagent.

Cetrimide R.
Contains not less than 96.0% and not more than 101.0% of alkyltrimethylammonium bromide, calculated as $C_{17}H_{38}BrN$ with reference to the dried substance.
Description. A white or almost white, voluminous, free-flowing powder; slight characteristic odor.
Solubility. Soluble in two parts of water; freely soluble in ethanol (~750 g/l) TS. A suitable commercially available reagent.

Chinese ink TS. Indian ink.
A suitable commercially available reagent.
Note. Before use, dilute 1 mL of black Chinese ink TS with 2 mL of water; if necessary, further dilute up to 1:10. It must be freshly prepared.

Chloral hydrate R. $C_2H_3Cl_3O_2$.
Description. Colorless, hygroscopic crystals with a sharp odor. Melting temperature. About 55°C.
A suitable commercially available reagent.

Chloral hydrate TS.
Procedure. Dissolve 50 g of chloral hydrate R in 20 mL of water.

Chloroform R. $CHCl_3$.
A suitable commercially available reagent.

Chromic acid TS.
Procedure. Dissolve 84 g of chromium trioxide R in 700 mL of water and add slowly, with stirring, 400 mL of sulfuric acid (~1760 g/l) TS.

Chromium trioxide R. CrO_3.
A suitable commercially available reagent.

Copper (II) sulfate R. $CuSO_4,5H_2O$.
A suitable commercially available reagent.

Crystal violet R. $C_{25}H_{30}ClN_3$.
A suitable commercially available reagent.

Cuoxam TS.
Tetramine copper dihydroxide, Schweizer's reagent.
Procedure. Dissolve 10 g of copper(II) sulfate R in 100 mL of water, and add sufficient sodium hydroxide (~240 g/l) TS until alkaline. Filter the precipitate and wash with cold water, previously made sulfate-free. To the moist copper hydroxide add, while stirring, ammonia (~100 g/l) TS until dissolved.
Note. Cuoxam TS must be freshly prepared.

Cyclohexane R. C_6H_{12}.
A suitable commercially available reagent.

Desmetryn R. $C_9H_{17}N_5S$.
2-methylmercapto-4-methylamino-6-isopropylamino-S- triazine.
A commercially available reagent suitable for use as a reference material.

Dichloromethane R. Methylene chloride, CH_2Cl_2.
Description. A clear colorless, mobile liquid.
Miscibility. Freely miscible with ethanol (~750 g/l) TS and ether R.
Boiling range. Not less than 95% distils between 39 and 41 °C.
Residue on evaporation. After evaporation on a water-bath and drying at 105 °C, leaves not more than 0.5 mg/mL.
A suitable commercially available reagent.

Dimethyl sulfoxide R. C_2H_6OS.
Description. A colorless liquid; odorless or with a slight unpleasant odor. Mass density (ρ20). 1.10 kg/l.
A suitable commercially available reagent.

Dimethyl yellow R. C.I. 11020; 4-dimethylaminoazobenzene; $C_{14}H_{15}N_3$.
Caution. Dimethyl yellow R is carcinogenic.
Description. Produces a red color in moderately acidic alcoholic solutions and yellow color in weakly acidic and alkaline solutions.
Homogeneity. Carry out the method for thin-layer chromatography, using silica gel G as the coating substance and dichloromethane R as the mobile phase. Apply 10 µl of a 0.1 mg/mL solution in dichloromethane R. After removing the plate from the chromatographic chamber allow it to dry in air. Only one spot appears on the chromatogram.
A suitable commercially available reagent.

Dioxan R. 1,4-Dioxane, $C_4H_8O_2$.
Caution. It is dangerous to determine the boiling range or the residue on evaporation before ensuring that the reagent complies with the test for peroxides, described below.
Description. A clear, colorless liquid.
Miscibility. Miscible with water, ethanol (~750 g/l) TS and ether R.
Boiling range. Not less than 95% distils between 101 and 105°C.
Melting temperature. Solidifies when cooled in ice and does not completely remelt at temperatures below 10°C.
Residue on evaporation. Evaporate on a water-bath and dry to constant weight at 105°C; leaves a residue of not more than 0.1 mg/mL.
Mass density (ρ_{20}). About 1.031 kg/l.
Water. Determined by the Karl Fischer method, not more than 5.0 mg/mL. Peroxides. Add 5 mL to a mixture of 1 g of potassium iodide R dissolved in 10 mL of water, 5 mL of hydrochloric acid (~70 g/l) TS, and 2 mL of starch TS, and mix; not more than a faint blue or brown color is produced.
A suitable commercially available reagent.

Dipotassium hydrogen phosphate R. K_2HPO_4.
A suitable commercially available reagent.

Disodium edetate R. $C_{10}H_{14}N_2Na_2O_8.2H_2O$.
A suitable commercially available reagent.

Disodium edetate (0.05 mol/l) VS. Disodium edetate R, dissolved in water to contain 16.81 g of $C_{10}H_{14}N_2Na_2O_8$ in 1000 mL.
Method of standardization. Ascertain the exact concentration by an appropriate method. The following method is suitable: transfer about 200 mg of calcium carbonate R2, accurately weighed, to a 400-mL beaker, add 10 mL of water, and swirl to form a slurry. Cover the beaker with a watch glass, and introduce 2 mL of hydrochloric acid (~70 g/l) TS from a pipette inserted between the lip of the beaker and the edge of the watch glass. Swirl the contents of the beaker to dissolve the calcium carbonate. Wash down the sides of the beaker, the outer surface of the pipette, and the watch glass with water, and dilute with water to about 100 mL. While stirring the solution, preferably with a magnetic stirrer, add about 30 mL of the disodium edetate solution from a 50-mL burette. Add 10 mL of sodium hydroxide (~80 g/l) TS and 0.3 g of calcon indicator mixture R or of calcon carboxylic acid indicator mixture R and

continue the titration with the disodium edetate solution to a blue end-point. Each 5.005 mg of calcium carbonate is equivalent to 1 mL of disodium edetate (0.05 mol/l) VS.

Disodium hydrogen phosphate R. $Na_2HPO_4.12H_2O$.
A suitable commercially available reagent.

Dragendorff's reagent
Solution A: Mix 10mL acetic acid and 40mL water and add 0.85g of bismuth (III) nitrate. Stir until dissolved. Label as solution A. Dissolve 8g potassium iodide in 20mL water. Label as solution B. Mix equal parts of Solution A and Solution B. Label as stock solution. Prepare a mixture of 2mL acetic acid and 10mL water then add 1mL of the stock solution. Label as spray reagent.

Equimolal phosphate, 0.05 m
Dissolve 3.53 g of disodium hydrogen phosphate (Na2HPO4) and 3.39 g of potassium dihydrogen phosphate (KH2PO4), each previously dried at 120° for 2 h, and dilute with water to make 1000 mL.

Ethanol R.
A suitable commercially available reagent.

Ethanol (~750 g/l) TS.
A suitable commercially available reagent.

Ethanol (~710g/l) TS.
A solution of about 950 mL of ethanol (~750 g/l) TS diluted with water to 1000 mL.

Ethanol (~375 g/l) TS.
A solution of about 525 mL of ethanol (~750 g/l) TS diluted with water to 1000 mL.

Ethanol (~188 g/l) TS.
A solution of about 260 mL of ethanol (~750 g/l) TS diluted with water to 1000 mL.

Ethanol (~150g/l) TS.
A solution of about 210 mL of ethanol (~750 g/l) TS diluted with water to 1000 mL.

Ether R. $C_4H_{10}O$.
A suitable commercially available reagent.

Ether/light petroleum TS1.
Procedure. Dilute 60 mL of ether R with sufficient distilled light petroleum R to produce 1000 mL.

Ether/light petroleum TS2.
Procedure. Dilute 150 mL of ether R with sufficient distilled light petroleum R to produce 1000 mL.

Ether/light petroleum TS3.
Procedure. Dilute 500 mL of ether R with sufficient distilled light petroleum R to produce 1000 mL.

Ethyl acetate R. $C_4H_8O_2$.
A suitable commercially available reagent.

Fast yellow R. C.I. 13015; E105; $C_{12}H9N_3Na_2O_6S_2$.
Description. An orange-yellow to red powder.
Solubility. Freely soluble in water; slightly soluble in ethanol (~750 g/l) TS; practically insoluble in ether R and chloroform R.
A suitable commercially available reagent.

Ferric ammonium sulfate R. $FeH_4NO_8S_2.12H_2O$.
This reagent should be free of chlorides.
A suitable commercially available reagent.

Ferric ammonium sulfate (0.25 mol/l) VS.
Ferric ammonium sulfate R, dissolved in nitric acid (~750 g/l) TS to contain 120.5 g of $FeH_4NO_8S_2.12H_2O$ in 1000 mL. Procedure. Dissolve 120.5 g of ferric ammonium sulfate R in a sufficient quantity of nitric acid (~750 g/l) TS to produce 1000 mL. The reagent should be free of chlorides.

Ferric chloride R. $FeCl_3,6H_2O$.
A suitable commercially available reagent.
Ferric chloride (50g/l) TS.
Procedure. Dissolve 5 g of ferric chloride R in 100 mL of water.

Ferrous sulfate. $FeSO_4.7\ H_2O$
A suitable commercially available reagent.

Florisil R.
A suitable commercially available material for column chromatography.
Formic acid (~1080 g/l) TS. CH_2O_2; *d* ~ 1.2. A suitable commercially available reagent.

D-Fructose R. $C_6H_{12}O_6$.
Description. A white, crystalline powder.
Melting point. About 103°C with decomposition.
Specific optical rotation. Use a 0.10 g/mL solution in water containing 0.05 mL of 20°C ammonia (~100 g/l) TS; [α]D = about -92°. A suitable commercially available reagent.

D-Galactose R. $C_6H_{12}O_6$.
Description. A white, crystalline powder.
Melting point. About 164°C.
Specific optical rotation. Use a 0.10 g/mL solution in water; [α]D
A suitable commercially available reagent.

Glucose hydrate R. Monohydrate of α-D'glucopyranose, $C_6H_{12}O_6.H_2O$. Contains not less than 99.0% and not more than 101.5% of $C_6H_{12}O_6$, calculated with reference to the dried substance.
Description. Colorless crystals or a white crystalline or granular powder; odorless. Solubility. Soluble in about 1 part of water and in about 60 parts of ethanol (~750 g/l) TS; more soluble in boiling water and in boiling ethanol (~750 g/l) TS.
Acidity. Dissolve 5 g in 50 mL of carbon-dioxide-free water R. Neutralization requires not more than 0.5 mL of carbonate-free sodium hydroxide (0.02 mol/l) VS, phenolphthalein/ethanol TS being used as indicator.
Specific optical rotation. Dissolve 100 mg, previously dried to constant weight, in 1 20°C mL of water, and add a few drops of ammonia (~100 g/l) TS; [α]D = + 52 to + 53°. Soluble starch or sulfites. Dissolve 1 g in 10 mL of water and add 1 drop of iodine TS; the liquid is colored yellow.
Loss on drying. Dry to constant weight at 105 °C; loses not less than 80 mg/g and not more than 100 mg/g.
Sulfated ash. Not more than 1.0 mg/g.
Assay. Dissolve about 0.1 g, accurately weighed, in 50 mL of water, add 30 mL of iodine (0.1 mol/l) VS and 10 mL of sodium carbonate (50 g/l) TS, and allow to stand for 20 min. Add 15 mL of hydrochloric acid (~70 g/l) TS and titrate the excess of iodine with sodium thiosulfate (0.1 mol/l) VS, using starch TS as indicator. Perform a blank determination and make any necessary corrections. Each mL of iodine (0.1 mol/l) VS is equivalent to 9.008 mg of $C_6H_{12}O_6$.

Glycerol R. Propane- 1,2,3-triol with small amounts of water, $C_3H_8O_3$. Contains not less than 970 g/kg of $C_3H_8O_3$.
Description. A clear, almost colorless, syrupy and hygroscopic liquid; odorless.

Miscibility. Miscible with water and ethanol (~750 g/l) TS; practically immiscible with ether R and chloroform R.
Mass density (ρ_{20}). Not less than 1.256 kg/l.
Refractive index (nD^{20}). Not less than 1.469.
Acrolein and other reducing substances. Mix 1 mL with 1 mL of ammonia (~100 g/l) TS and heat in a water-bath at 60 °C for 5 min; the liquid is not colored yellow. Remove from the water-bath and add 3 drops of silver nitrate (40 g/l) TS; the liquid does not become colored within 5 min.
Sulfated ash. Not more than 0.5 mg/mL.
A suitable commercially available reagent.

Glycerol-ethanol TS.
Procedure. Mix equal volumes of glycerol R, water and ethanol (~750 g/l) TS. Glycine R. Aminoacetic acid, $C_2H_5NO_2$.
A suitable commercially available reagent.

Helium R. He.
Contains not less than 999.95 mL of He per litre. A suitable commercially available reagent.

Helium for chromatography. He.
Contains not less than 99.995 per cent *V/V* of He. A suitable commercially available reagent.

Hexane. C_6H_{14}.
A colorless, flammable liquid, practically insoluble in water, miscible with ethanol and with ether.
d^{20}: 0.659 to 0.663. 20
nD^{20}: 1.375 to 1.376
Distillation range. Not less than 95 percent distils between 67 °C and 69 °C. A suitable commercially available reagent.

Hexane R. n-Hexane, C_6H_{14}.
Description. A colorless, mobile, highly inflammable liquid.
Boiling range. Distills completely over a range of 1 °C between 67.5 and 69.5 °C.
Mass density (ρ_{20}). 0.658–0.659 kg/l.
Refractive index (nD^{20}). 1.374–1.375.
A suitable commercially available reagent.

Hide powder R.
A suitable commercially available reference material.

Hydrochloric acid R. HCl
A suitable commercially available reagent.
Hydrochloric acid (1 mol/l) VS. Hydrochloric acid (~250 g/l) TS, diluted with water to contain 36.47 g of HCl in 1000 mL.
Method of standardization. Ascertain the exact concentration of the 1 mol/l solution in the following manner. Dissolve about 1.5 g, accurately weighed, of anhydrous sodium carbonate R (previously dried at 270 °C for 1 hour) in 50 mL of water and titrate with the hydrochloric acid solution, using methyl orange/ethanol TS as indicator. Each 52.99 mg of anhydrous sodium carbonate R is equivalent to 1 mL of hydrochloric acid (1 mol/l) VS.

Hydrochloric acid (~420 g/l) TS. *d* ~1.18.
A suitable commercially available reagent.

Hydrochloric acid (~ 250 g/l) AsTS.
Hydrochloric acid (~250 g/l) TS that complies with the following tests A and B.
A. Dilute 10 mL with sufficient water to produce 50 mL, add 5 mL of ammonium thiocyanate (75 g/l) TS and stir immediately; no color is produced.
B. To 50 mL add 0.2 mL of bromine AsTS, evaporate on a water-bath until reduced to 16 mL, adding more bromine AsTS if necessary to ensure that an excess, as indicated by the color, is present throughout the evaporation. Add 50 mL of water and 5 drops of stannous chloride AsTS and apply the general test for arsenic. The color of the stain

produced is not more intense than that produced from a 0.2-mL standard stain, showing that the amount of arsenic does not exceed 0.05 µg/mL.
Hydrochloric acid (~ 250 g/l) TS. A solution of hydrochloric acid (~420 g/l) TS in water, containing approximately 250 g of HCl per liter; *d* ~1.12.

Hydrochloric acid (~ 250 g/l), stannated, AsTS.

Procedure. Dilute 1 mL of stannous chloride AsTS with sufficient hydrochloric acid (~250 g/l) AsTS to produce 100 mL.

Hydrochloric acid (~ 70 g/l) TS.

Procedure. Dilute 260 mL of hydrochloric acid (~250 g/l) TS with sufficient water to produce 1000 mL (approximately 2 mol/l); *d* ~1.035.

Hydrochloric acid, dilute, R

Dilute 23.6 mL of hydrochloric acid with water to make 100 mL (10%).

Hydrogen for chromatography. H_2.

Contains not less than 99.95 percent *V/V* of H_2. A suitable commercially available reagent.

Indian ink

see Chinese ink TS.

Indophenol blue R. C.I. 49700; $C_{18}H_{26}N_2O$.

Description. A violet-black powder.
Solubility. Practically insoluble in water; soluble in chloroform R.
Homogeneity. Carry out the method for thin-layer chromatography, using silica gel G as the coating substance and dichloromethane R as the mobile phase. Apply 10 µl of a 0.1 mg/mL solution in dichloromethane R and develop. After removing the plate from the chromatographic chamber allow it to dry in air. Only one spot appears on the chromatogram.
A suitable commercially available reagent.

Iodine R. I_2.

A suitable commercially available reagent.

Iodine TS.

Procedure. Dissolve 2.6 g of iodine R and 3 g of potassium iodide R in sufficient water to produce 100 mL (approximately 0.1 mol/l).

Iodine (0.1 mol/l) VS.

Iodine R and potassium iodide R, dissolved in water to contain 25.38 g of I2 and 36 g of KI in 1000 mL.
Method of standardization. Ascertain the exact concentration of the 0.1 mol/l solution by titrating 25.0 mL with sodium thiosulfate (0.1 mol/l) VS, using starch TS as indicator.

Iodine (0.02 mol/l) VS.

Iodine R and potassium iodide R, dissolved in water to contain 5.076 g of I2 and 7.2 g of KI in 1000 mL.
Method of standardization. Ascertain the exact concentration of the solution following the method described under iodine (0.1 mol/l) VS.

Iodine/ethanol TS.

Procedure. Dissolve 10 g of iodine R in sufficient ethanol (~750 g/l) TS to produce 1000 mL. Iron(III) citrate R. Ferric citrate, $C_6H_5FeO_7.H_2O$.
A suitable commercially available reagent.

Lactic acid R. $C_3H_6O_3$.
Quality conforms to the monograph in The international pharmacopeia (5).

Lactochloral TS.
Procedure. Dissolve 50 g of chloral hydrate R in 50 g of lactic acid R by gentle heating.

Lactophenol TS.
Procedure. To a mixture of 20 g of lactic acid R and 40 g of glycerol R dissolved in 20 mL of water, add 20 g of phenol R and mix.

Lactose R. $C_{12}H_{22}O_{11}$.
A suitable commercially available reagent.

Lead acetate R. $C_4H_6O_4Pb.3H_2O$.
A suitable commercially available reagent.

Lead acetate (80 g/l) TS.
A solution of lead acetate R in freshly boiled water containing about 80 g/l of $C_4H_6O_4Pb$ (approximately 0.25 mol/l).

Lead(II) acetate TS.
To 9.5 g of lead(II) acetate trihydrate, add freshly boiled and cooled water to make 100 mL. Preserve in tightly stoppered bottles (0.25 mol/l).

Lithium chloride R. LiCl.
Description. White, deliquescent crystals or granules.
Solubility. Freely soluble in water; soluble in acetone R and ethanol (~ 750 g/l) TS. Storage. Store in a tightly closed container.
A suitable commercially available reagent.

L-Lysine R. $C_6H_{14}N_2O_2$.
Description. Crystalline needles or hexagonal plates.
Solubility. Soluble in water; very slightly soluble in ethanol (~750 g/l) TS; insoluble in ether R.
Melting point. About 213 °C with decomposition.
Specific optical rotation. Dissolve 0.2 g in 10 mL of hydrochloric acid (~250 g/l) TS; [α]D = about +21.5 °.
A suitable commercially available reagent.

Magnesium chloride R. $MgCl_2.6H_2O$.
A suitable commercially available reagent.

Magnesium acetate, 0.5%
Dissolve 0.5 g magnesium acetate in sufficient methanol TS to produce 100 mL solution. In testing for anthraquinones, heat the sprayed TLC plates at 90°C for 5 mins.

Magnesium nitrate R. $Mg(NO_3)_2$
See Magnesium nitrate hexahydrate.

Magnesium nitrate hexahydrate R. $Mg(NO_3)_2.6H_2O$
A suitable commercially available reagent.

Mercuric bromide R. $HgBr_2$.
A suitable commercially available reagent.

Mercuric bromide AsTS.
Procedure. Dissolve 5 g of mercuric bromide R in sufficient ethanol (~ 750 g/l) TS to produce 100 mL.

Mercuric bromide paper AsR.
Procedure. Use smooth, white filter paper weighing 65–120 g/m2. The thickness of the paper in mm should be approximately equal numerically to the weight expressed as above, divided by 400. Soak pieces of filter paper, not less than 25 mm in width, in mercuric bromide AsTS, decant the superfluous liquid, suspend the paper over a non-metallic thread and allow it to dry, protected from light.
Storage. Store the mercuric bromide paper AsR in stoppered bottles in the dark. Note. Paper that has been exposed to sunlight or to vapors of ammonia must not be used as it produces only a pale stain or no stain at all.

Mercuric nitrate TS.
Millon's reagent; nitric acid solution of mercury.
Procedure. Dissolve 1 mL of mercury R in 9 mL of fuming nitric acid R, keeping the mixture well cooled during the reaction. When the reaction is complete, dilute the solution with an equal volume of water. It should be protected from light and used within two months of preparation.

Mercuric thiocyanate R. $C_2HgN_2S_2$.
A suitable commercially available reagent.

Mercuric thiocyanate TS.
A saturated solution of mercuric thiocyanate R in ethanol (~ 750 g/l) TS.

Mercury R. Hg.
A suitable commercially available reagent.

Methane R. CH_4.
A suitable commercially available reagent.

Methanol R. CH_4O.
A suitable commercially available reagent.

Methyl laurate R. $C_{13}H_{26}O_2$.
Description. A colorless or pale yellow liquid.
Mass density (ρ_{20}). About 0.87 kg/l.
A suitable commercially available reagent.

Methyl myristate R. $C_{15}H_{30}O_2$.
Description. A colorless or slightly yellow liquid.
Mass density (ρ_{20}). About 0.87 kg/l.
A suitable commercially available reagent.

Methyl orange R. Sodium salt of 4'-dimethylaminoazobenzene-4-sulfonic acid, $C_{14}H_{14}N_3NaO_3S$.
A suitable commercially available reagent.

Methyl orange TS.
Dissolve 0.1 g of methyl orange in 100 mL of water, and filter if necessary.

Methyl orange/ethanol TS.
Procedure. Dissolve 0.04 g of methyl orange R in sufficient ethanol (~ 150 g/l) TS to produce 100 mL.

Methyl palmitate R. Methyl hexadecanoate; $C_{17}H_{34}O_2$.
Description. A colorless, waxy solid.
Freezing point. About 27°C.
Mass density (ρ_{20}). About 0.86 kg/l.
A suitable commercially available reagent.

Methyl red. C.I. 13020. 2-(4-Dimethylamino-phenylazo)benzoic acid. $C_{15}H_{15}N_3O_2$.
A dark-red powder or violet crystals, practically insoluble in water, soluble in alcohol.
A suitable commercially available reagent.

Methyl stearate R. $C_{19}H_{38}O_2$.
Description. A white or pale yellow, crystalline mass. Melting point. About 38°C.
A suitable commercially available reagent.

1-Naphthol R. $C_{10}H_8O$.
Description. Colorless crystals or a white, crystalline powder; odor, characteristic.
Solubility. Soluble in 5 parts of ethanol (~750 g/l) TS (may form a slightly opalescent, colorless or almost colorless solution).
Melting range. 93-96°C.
Sulfated ash. Not more than 0.5 mg/g.
A suitable commercially available reagent.

1-Naphthol TS.
Procedure. Dissolve 20 g of 1-naphthol R in 100 mL of ethanol (~750 g/l) TS. Protect from light and use within a few days of preparation.

Neutral red R. C.I. 50040; C.I. Basic red; $C_{15}H_{17}ClN_4$.
A suitable commercially available reagent.

Nitric acid, fuming, R. HNO_3.
A suitable commercially available reagent.

Nitric acid (~ 1000 g/l) TS. *d* ~ 1.41.
A suitable commercially available reagent.

Nitric acid (~ 750 g/l) TS.
Procedure. Dilute 750 mL of nitric acid (~1000 g/l) TS with sufficient water to produce 1000 mL (approximately 12 mol/l).

Nitric acid (~500 g/l) TS.
Procedure. Dilute 500 mL of nitric acid (~1000 g/l) TS with sufficient water to produce 1000 mL (approximately 8 mol/l).

Nitric acid (~225 g/L) TS.
Procedure. Dilute 225 mL of nitric acid (~1000 g/L) TS with sufficient water to produce 1000 mL (approximately 3.5 mol/l).

Nitric acid R. HNO_3 (concentration: 69–70%, density: about 1.42 g/mL)
A suitable commercially available reagent.

Nitro-chromic acid TS.
Procedure. Mix equal volumes of nitric acid (~225 g/L) TS and chromic acid TS.

Nitrogen. N_2.
Nitrogen, washed and dried.
A suitable commercially available reagent.

Nitrogen for chromatography.
Contains not less than 99.95 per cent *V/V* of N2. A suitable commercially available reagent.

Nitrogen, oxygen-free.
Nitrogen R which has been freed from oxygen by passing it through alkaline pyrogallol solution R.

Nitrogen R. N_2.
A suitable commercially available reagent.

Oracet blue 2R. CI 61110. 1-amino-4-(phenylamino)anthracene-9,10-dione. $C_{20}H_{14}N_2O_2$. mp: about 194 °C.
A suitable commercially available reagent.

Ox bile, dehydrated, R.
Dehydrated, purified fresh bile. A suitable commercially available reagent.

Pancreatic digest of casein R.
A suitable commercially available reagent.

Pancreatic digest of gelatin R.
A suitable commercially available reagent.

Papaic digest of soybean meal R.
A suitable commercially available reagent.

Paraffin, liquid, R.
A suitable commercially available reagent.

Peptone, dried, R.
A variety of peptones are available from casein, meat, beef or a mixture of these.
A suitable commercially available reagent.

Perchloric acid (~ 1170 g/l) TS. *d* ~ 1.67.
A suitable commercially available reagent.

Petroleum, light, R.
A suitable commercially available reagent.

Phenol R. C_6H_6O
Description. Colorless, or at most faintly pink, cohering or separate acicular crystals, or crystalline masses; characteristic odor. Corrosive, and blanches the skin and mucous membranes.
Solubility. Soluble in about 15 parts of water and in about 100 parts of liquid paraffin R; freely soluble in ethanol (~750 g/L) TS, ether R and chloroform R.
Completeness of solution. 1 g dissolves completely in 15 mL of water at 15°C.
Congealing temperature. Not below 40.5°C.
Residue on evaporation. Evaporate on a water-bath and dry to constant weight at 105°C; leaves not more than 0.5 mg/g of residue.
A suitable commercially available reagent.

Phenolphthalein R. $C_{20}H_{14}O_4$.
A suitable commercially available reagent.

Phenolphthalein TS.
Dissolve 1 g of phenolphthalein in 100 mL of ethanol R.

Phenolphthalein/ethanol TS.
Procedure. Dissolve 1 g of phenolphthalein R in sufficient ethanol (~ 750 g/l) TS to produce 100 mL.

Phenol red R. Phenolsulfonphthalein, $C_{19}H_{14}O_5S$.
A suitable commercially available reagent.

Phenol red/ethanol TS.
Procedure. Dissolve 0.05 g of phenol red R in a mixture of 2.85 mL of sodium hydroxide (0.05 mol/L) VS and 5 mL of ethanol (~710 g/L) TS. Warm the solution slightly and after cooling dilute with sufficient ethanol (~150 g/L) TS to produce 250 mL.

Phloroglucinol R. Benzene-1,3,5-triol dihydrate; $C_6H_6O_3,2H_2O$.
Description. White or pale cream crystals.
Melting point. About 220°C.
A suitable commercially available reagent.

Phloroglucinol TS.
Procedure. Dissolve 1 g of phloroglucinol R in 100 mL of ethanol (~750 g/L) TS.

Phosphate buffer, pH 7.4, TS.
Procedure. Dissolve 6.8 g of potassium dihydrogen phosphate R in 250 mL of water and add 393.4 mL of sodium hydroxide (0.1 mol/L) VS.

Phosphate buffer solution, pH 7.2.
Mix 50 mL of 0.2 mol/L potassium dihydrogen phosphate TS for buffer solution and 34.7 mL of 0.2 mol/L sodium hydroxide VS, and add water to make 200 mL.

Phosphomolybdic acid R. $H_3PO_4,12MoO_3,24H_2O$.
A suitable commercially available reagent.

Phosphorus pentoxide R. P_2O_5.
A suitable commercially available reagent.

Poly(dimethyl)(diphenyl)siloxane.
Contains 95% of methyl groups and 5% of phenyl groups. DB-5, SE52. Stationary phase for gas chromatography.
A suitable commercially available material for column chromatography.

Poly(dimethyl)siloxane.
Silicone gum rubber (methyl). Organosilicon polymer with the appearance of a semi-liquid, colorless gum.
A suitable commercially available material for column chromatography.

Polysorbate 20 R.
Quality conforms to the monograph in *The international pharmacopeia (5)*.

Polysorbate 80 R.
Quality conforms to the monograph in *The international pharmacopeia (5)*.

Potassium biphthalate, 0.05 m
Dissolve 10.12 g of $KHC_8H_4O_4$, previously dried at 110°C for 1 h, and dilute with water to make 1000 mL.

Potassium bromide R. KBr.
A suitable commercially available reagent.

Potassium chlorate R. $KClO_3$.
A suitable commercially available reagent.

Potassium dichromate R. $K_2Cr_2O_7$.
A suitable commercially available reagent.

Potassium dichromate (0.0167 mol/L) VS.
Potassium dichromate R, dissolved in water to contain 4.904 g of $K_2Cr_2O_7$ in 1000 mL.
Method of standardization. Ascertain the exact concentration of the 0.0167 mol/L solution in the following manner: Place 25.0 mL of the potassium dichromate solution into a glass-stoppered flask. Add 2 g of potassium iodide R, dilute with 200 mL of water, add 5 mL of hydrochloric acid (~420 g/L) TS, allow to stand for 10 min in a dark place, and titrate the liberated iodine with sodium thiosulfate (0.1 mol/L) VS, adding 3 mL of starch TS as the end-point is approached. Correct for a blank determined using the same quantities of the same reagents.

Potassium dihydrogen citrate, 0.05 M
Dissolve 11.41 g of C6H7KO4, and dilute with water to make 1000 mL.

Potassium dihydrogen phosphate R. KH_2PO_4.
A suitable commercially available reagent.

Potassium dihydrogen phosphate, 0.0087 M and disodium hydrogen phosphate, 0.0303 M
Dissolve 1.18 g of KH2PO4 and 4.30 g of Na2HPO4, both dried for 2 h at 120 ± 2°, and dilute with water to make 1000 mL.

Potassium dihydrogen phosphate TS (0.2 mol/L)
Dissolve 27.22 g of potassium dihydrogen phosphate in water to make 1000 mL.

Potassium hydrogen phthalate R. $C_8H_5KO_4$.
A suitable commercially available reagent.

Potassium hydrogen tartrate, saturated at 25°
Add $C_4H_5KO_6$ to water until saturation is exceeded at 25°. Then filter or decant.

Potassium hydroxide. KOH
A suitable commercially available reagent.

Potassium hydroxide R. KOH.
A suitable commercially available reagent.

Potassium hydroxide (~110 g/l) TS.
A solution of potassium hydroxide R containing about 112 g of KOH per liter (approximately 2 mol/L).

Potassium hydroxide (~55 g/l) TS.
A solution of potassium hydroxide R containing about 56 g of KOH per liter (approximately 1 mol/L).

Potassium iodide R. KI.
A suitable commercially available reagent.

Potassium iodide TS.
Dissolve 16.5 g of potassium iodide in water to make 100 mL. Preserve in light-resistant containers. Prepare before use (1 mol/l).

Potassium iodide AsR.
Potassium iodide R that complies with the following test: Dissolve 10 g of potassium iodide R in 25 mL of hydrochloric acid (~ 250 g/l) as TS and 35 mL of water, add 2 drops of stannous chloride AsTS and apply the general test for arsenic; no visible stain is produced.

Potassium iodide (80 g/l) TS.
A solution of potassium iodide R containing about 83 g of KI per liter (approximately 0.5 mol/l).

Potassium sulfate R. K_2SO_4.
A suitable commercially available reagent.

Potassium tellurite R. K_2TeO_3 (approx.)
A suitable commercially available reagent.

Potassium tetraoxalate, 0.05 m
Dissolve 12.61 g of KH3(C2O4)2 · 2H2O, and dilute with water to make 1000 mL.

Potassium tetrathionate R. $K_2S_4O_6$.
A suitable commercially available reagent.

Prometryn R. $C_{10}H_{19}N_5S$.
A commercially available reagent suitable for use as a reference material.

1-Propanol R. n-Propanol; propan-1-ol, C_3H_8O.
Description. A clear, colorless liquid.
Miscibility. Miscible with water and ethanol (~750 g/l) TS. Boiling range. Not less than 95% distils between 95 and 98°C. Mass density (ρ20). About 0.803 kg/l.
Residue on evaporation. Evaporate on a water-bath and dry to constant weight at 105°C; leaves a residue of not more than 0.1 mg/g.
A suitable commercially available reagent.

2-Propanol R. Isopropyl alcohol; C_3H_8O.
A suitable commercially available reagent.

Propylene glycol R. Propane diol, $C_3H_8O_2$.
A suitable commercially available reagent.

Pyridine R. C_5H_5N.
A suitable commercially available reagent.

Pyrogallol R. $C_6H_6O_3$. Benzene-1,2,3-triol.
White crystals, becoming brownish on exposure to air and light, very soluble in water, in alcohol and in ether, slightly soluble in carbon disulfide. On exposure to air, aqueous solutions, and more rapidly alkaline solutions, become brown owing to the absorption of oxygen. mp: about 131 °C.
Store protected from light.
A suitable commercially available reagent.

Pyrogallol solution, alkaline.
Dissolve 0.5 g pyrogallol R in 2 mL of carbon dioxide-free water R. Dissolve 12 g of potassium hydroxide R in 8 mL of carbon dioxide-free water R. Mix the two solutions immediately before use.

Quinine hydrochloride R. $C_{20}H_{24}N_2O_2$,HCl,2H_2O.
Quality of substance conforms to the monograph in *The international pharmacopeia (5)*.

Saponin R.
A suitable commercially available reference material.

Silica gel G
Description. A fine, white, homogeneous powder with an average particle size of between 10 and 44 µm containing about 130 g of calcium sulfate, hemihydrate per kg.
Content of calcium sulfate. Place about 0.25 g, accurately weighed, in a flask with a ground-glass stopper, add 3 mL of hydrochloric acid (~ 70 g/l) TS and 100 mL of water and shake vigorously for 30 min. Filter through a sintered-glass filter and wash the residue. Using the combined filtrate and washings, carry out the assay for

calcium by complexometry (5). Each mL of disodium edetate (0.05 mol/l) VS is equivalent to 7.26 mg of $CaSO_4.1/2H_2O$ (MW 145.1).
pH. Shake 1 g with 10 mL of carbon-dioxide-free water R for 5 min. Measure the pH potentiometrically (5); pH is about 7.
Preparation. Suspend 30 g in 60 mL of water, shaking vigorously for 30 seconds. Carefully coat the cleaned plates with a layer 0.25 mm thick using a spreading device. Allow the coated plates to dry in air.
Separating power. Apply separately to the adsorbent layer 10 µl of 0.10 mg/mL solutions containing respectively indophenol blue R, Sudan red G R and dimethyl yellow R in toluene R. Develop the plate with the same solvent over a distance of 10 cm. The chromatogram shows three clearly separated spots, the spot of indophenol blue near the points of application, that of dimethyl yellow in the middle of the chromatogram, and that of Sudan red G between the two.

Silica gel R.
A suitable commercially available material for column chromatography.

Silica gel, desiccant, R.
Description. An amorphous, partly hydrated SiO_2, occurring in glassy granules of varying sizes. It is frequently coated with a substance that changes color when the capacity to absorb water is exhausted. Such colored products may be regenerated (i.e., may regain their capacity to absorb water) by heating at 110°C until the gel assumes the original color.
Loss on drying. Ignite 2 g, accurately weighed, at 950±50°C to constant weight; the loss is not more than 60 mg/g.
Water absorption. Place about 10g in a tared weighing-bottle, and weigh. Then place the bottle, with the cover removed, for 24 hours in a closed container in which 80% relative humidity is maintained by being in equilibrium with sulfuric acid having a relative density of 1.19. Weigh again; the increase in weight is not less than 310 mg/g.
A suitable commercially available reagent.

Silver nitrate R. $AgNO_3$.
A suitable commercially available reagent.

Silver nitrate (40 g/l) TS.
solution of silver nitrate R containing about 42.5 g of $AgNO_3$ per litre (approximately 0.25 mol/l).

Simazine R. $C_7H_{12}ClN_5$.
A commercially available reagent suitable for use as a reference material.

Soda lime R.
A suitable commercially available reagent.

Sodium acetate R. $C_2H_3NaO_2,3H_2O$.
A suitable commercially available reagent.

Sodium acetate (1.6g/l) TS.
A solution of sodium acetate R containing about 1.64 g of $C_2H_3NaO_2$ per litre (0.02 mol/l).

Sodium carbonate, 0.025 M and sodium bicarbonate, 0.025 M
Dissolve 2.64 g of sodium carbonate (Na2CO3) and 2.09 g of sodium bicarbonate (NaHCO3), and dilute with water to make 1000 mL.

Sodium carbonate R. $Na_2CO_3,10H_2O$.
A suitable commercially available reagent.

Sodium carbonate, anhydrous, R. Na_2CO_3.
A suitable commercially available reagent.

Sodium carbonate (50 g/l) TS.
A solution of sodium carbonate R containing about 50 g of Na_2CO_3 per litre (approximately 0.5 mol/l).

Sodium chloride R. NaCl.
A suitable commercially available reagent.

Sodium chloride (400 g/l) TS.
A solution of sodium chloride R containing about 400 g of NaCl per litre.

Sodium chloride (100g/l) TS.
A solution of sodium chloride R containing about 100 g of NaCl per litre.

Sodium citrate R. $C_6H_5Na_3O_7.2H_2O$.
Quality of substance conforms to the monograph in The international pharmacopeia (5).

Sodium citrate (36.5 g/l) TS.
A solution of sodium citrate R containing about 36.5 g of $C_6H_5Na_3O_7$ per litre.

Sodium deoxycholate R. $C_{23}H_{39}NaO_4$.
Containing not less than 90% of $C_{23}H_{39}NaO_4$. A suitable commercially available reagent.

Sodium hydroxide R. NaOH.
A suitable commercially available reagent.

Sodium hydroxide (~ 240 g/l) TS.
A solution of sodium hydroxide R containing about 240 g of NaOH per litre of carbon-dioxide-free water R.

Sodium hydroxide (~80g/l) TS.
A solution of sodium hydroxide R containing about 80 g of NaOH per litre (approximately 2 mol/l).

Sodium hydroxide (1 M).
Dissolve 162 g of sodium hydroxide in 150 mL of carbon dioxide-free water R, cool the solution to room temperature and filter through hardened filter paper. Dilute 54.5 mL of the clear filtrate with carbon dioxide-free water R to 1000 mL. Standardization. weigh accurately about 5 g of potassium hydrogen phthalate, previously crushed lightly and dried at 120 °C for 2 hours, and dissolve in 75 mL of carbon dioxide-free water R. Add 0.1 mL of phenolphthalein TS, and titrate with the sodium hydroxide solution to the production of a permanent pink color. Each 204.2 mg of potassium hydrogen phthalate is equivalent to 1 mL of 0.1 M sodium hydroxide VS.
Storage. Solutions of alkali hydroxides absorb carbon dioxide when exposed to air. They should therefore be stored in bottles with suitable non-glass, well-fitting stoppers, provided with a tube filled with the soda lime.
Note. Prepare solutions at lower concentrations (e.g., 0.1 M, 0.01 M) by quantitatively diluting accurately measured volumes of the 1 M solution with sufficient carbon dioxide-free water R to yield the desired concentration. Restandardize the solution frequently.

Sodium hydroxide (1 mol/l) VS.
Sodium hydroxide R dissolved in water to produce a solution containing 40.01 g of NaOH in 1000 mL.
Method of standardization. Ascertain the exact concentration of the 1 mol/l solution in the following manner: dry about 5 g of potassium hydrogen phthalate R at 105 °C for 3 hours and weigh accurately. If the potassium hydrogen phthalate is in the form of large crystals, they should be crushed before drying. Dissolve in 75 mL of carbon-dioxide-free water R and titrate with the sodium hydroxide solution, using phenolphthalein/ethanol TS as indicator. Each 0.2042 g of potassium hydrogen phthalate is equivalent to 1 mL of sodium hydroxide (1 mol/l) VS. Standard solutions of sodium hydroxide should be restandardized frequently.
Storage. Solutions of alkali hydroxides absorb carbon dioxide when exposed to air. They should therefore be stored in bottles with suitable non-glass, tightly-fitting stoppers, provided with a tube filled with soda lime R.
Sodium hydroxide (0.5 mol/l) VS. Sodium hydroxide R dissolved in water to produce a solution containing 20.00 g of NaOH in 1000 mL.

Method of standardization. Ascertain the exact concentration of the solution following the method described under sodium hydroxide (1 mol/l) VS.

Sodium hydroxide (0.1 mol/l) VS.

Sodium hydroxide R dissolved in water to produce a solution containing 4.001 g of NaOH in 1000 mL.
Method of standardization. Ascertain the exact concentration of the solution following the method described under sodium hydroxide (1 mol/l) VS.

Sodium hydroxide (0.05 mol/l) VS.

Sodium hydroxide R, dissolved in water to produce a solution containing 2.000 g of NaOH in 1000 mL.
Method of standardization. Ascertain the exact concentration of the solution following the method described under sodium hydroxide (1 mol/l) VS.

Sodium hydroxide (0.02 mol/l), carbonate-free, VS.

Sodium hydroxide R, dissolved in water to produce a solution containing 0.8001 g of NaOH in 1000 mL.
Method of standardization. Ascertain the exact concentration of the solution following the method described under sodium hydroxide (1 mol/l) VS.

Sodium hydroxide, methanolic TS.

Procedure. Dissolve 2.5 g of sodium hydroxide R in 10 mL of carbon-dioxide-free water R. Add 1 mL of propylene glycol R and dilute to 100 mL with methanol R.

Sodium hypochlorite TS.

Containing 100-140 g of available chlorine per litre.
Description. A yellowish liquid; odor of chlorine. A suitable commercially available reagent.

Sodium metabisulfite R. $Na_2O_5S_2$.

A suitable commercially available reagent.

Sodium metabisulfite (150 g/l) TS.

A solution of sodium metabisulfite R containing about 150 g of $Na_2O_5S_2$ per litre.

Sodium pyruvate R. $C_3H_3NaO_2$.

Description. An almost white to white powder or a crystalline powder.
Solubility. Soluble in water.
A suitable commercially available reagent.

Sodium sulfate, anhydrous, R. Na_2SO_3.

A suitable commercially available reagent.

Sodium sulfide TS. Na_2S

Dissolve 5 g of sodium sulfide ennaehydrate in a mixture of 10 mL of water and 30 mL of glycerin. Or dissolve 5 g of sodium hydroxide in a mixture of 30 mL of water and 90 mL of glycerin, saturate half the volume of this solution with hydrogen sulfide, while cooling, and mix with the remaining half. Preserve in well-filled, light-resistant bottles. Use within 3 months.

Sodium sulfide ennaehydrate R. $Na_2S,9H_2O$.

A suitable commercially available reagent.

Sodium sulfite, anhydrous, R. Na_2SO_3

A suitable commercially available reagent.

Sodium tetraborate, 0.01 m

Dissolve 3.80 g of Na2B4O7 · 10H2O, and dilute with water to make 1000 mL. Protect from absorption of carbon dioxide.

Sodium tetrahydroborate R. $NaBH_4$.
Hygroscopic crystals, freely soluble in water, soluble in ethanol.

Sodium thiosulfate R. $Na_2S_2O_3,5H_2O$.
A suitable commercially available reagent.

Sodium thiosulfate (0.1 mol/l) VS.
Sodium thiosulfate R, dissolved in water to produce a solution containing 15.82 g of $Na_2S_2O_3$ in 1000 ml.
Method of standardization. Ascertain the exact concentration of the 0.1 mol/l solution in the following manner: transfer 30.0 mL of potassium dichromate (0.0167 mol/l) VS to a glass-stoppered flask and dilute with 50 mL of water. Add 2 g of potassium iodide R and 5 mL of hydrochloric acid (~250 g/l) TS, stopper and allow to stand for 10 min. Dilute with 100 mL of water and titrate the liberated iodine with the sodium thiosulfate solution, using starch TS as indicator. Sodium thiosulfate solutions should be restandardized frequently.

Stannous chloride R. $SnCl_2,2H_2O$.
A suitable commercially available reagent.

Stannous chloride TS.
Procedure. Dissolve 330 g of stannous chloride R in 100 mL of hydrochloric acid (~250 g/l) TS and sufficient water to produce 1000 mL.

Stannous chloride AsTS.
Procedure. Prepare from stannous chloride TS by adding an equal volume of hydrochloric acid (~250 g/l) TS, boiling down to the original volume, and filtering through a fine-grained filter paper.
Test for arsenic. To 10 mL add 6 mL of water and 10 mL of hydrochloric acid (~250 g/l) AsTS, and distil 16 mL. To the distillate add 50 mL of water and 2 drops of stannous chloride AsTS and apply the general test for arsenic. The color of the stain produced is not more intense than that produced from a 1-mL standard stain, showing that the amount of arsenic does not exceed 1 µg/mL.

Starch R.
A suitable commercially available reagent.

Starch, soluble, R.
A suitable commercially available reagent.

Starch TS.
Procedure. Mix 0.5 g of starch R or of soluble starch R with 5 mL of water, and add this solution, with constant stirring, to sufficient water to produce about 100 mL; boil for a few min, cool, and filter.
Note. Starch TS should be freshly prepared.

Styrene-divinylbenzene copolymer.
Porous, rigid, cross-linked polymer beads. Several grades are available with different sizes of beads. The size range of the beads is specified after the name of the reagent in the tests where it is used.
A suitable commercially available material.

Sucrose R. $C_{12}H_{22}O_{11}$.
A suitable commercially available reagent.

Sudan red G R. 1-(4-Phenylazophenylazo)-2-naphthol; sudan III; solvent red 23; C.I. 26100; $C_{22}H_{16}N_4O$.
Description. A reddish brown powder.
Solubility. Practically insoluble in water; soluble in chloroform R.
A suitable commercially available reagent.

Sudan red TS.
Procedure. Dissolve 0.5 g of sudan red G R in 100 mL of glacial acetic acid R.

Sulfuric acid R. H_2SO_4
A suitable commercially available reagent.

Sulfuric acid (~1760 g/l) TS. *d* ~ 1.84.
A suitable commercially available reagent.

Sulfuric acid (~1160 g/l) TS.
Procedure. Add 660 mL of sulfuric acid (~1760 g/l) TS to sufficient water to produce 1000 mL.

Sulfuric acid (~350 g/l) TS.
Procedure. Add 200 mL of sulfuric acid (~1760 g/l) TS to sufficient water to produce 1000 mL.

Sulfuric acid (~300 g/l) TS.
. Add 171 mL of sulfuric acid (~ 1760 g/l) TS to sufficient water to produce 1000 mL (approximately 3 mol/l).

Sulfuric acid (~ 37 g/l) TS.
Procedure. Add 21.5 mL of sulfuric acid (~ 1760 g/l) TS to sufficient water to produce 1000 mL (approximately 0.375 mol/l).

Tetracycline R. $C_{22}H_{24}N_2O_8$.
A suitable commercially available reagent.

N,N,N',N'-Tetramethyl-p-phenylenediamine dihydrochloride R. $C_{10}H_{16}N_2$, 2HCl.
Description. Whitish grey crystals.
A suitable commercially available reagent.

Thionine R. C.I. 52000; $C_{12}H_{10}ClN_3S$.
Description. Blackish green glistening crystals. Solubility. Freely soluble in hot water.
A suitable commercially available reagent.

Thionine TS.
Procedure. Dissolve 0.2 g of thionine R in 100 mL of ethanol (~188 g/l) TS.

Toluene R. C_7H_8. Methylbenzene.
A clear, colorless, flammable liquid, very slightly soluble in water, miscible with alcohol. d^{20}: 0.865 to 0.870. 20
bp: about 110 °C.
A suitable commercially available reagent.

Trifluoroacetic acid (TFA). CF_3COOH.
A suitable commercially available reagent.

2,2,4-Trimethylpentane R. C_8H_{18}.
A suitable commercially available reagent.

Trinitrophenol R. $C_6H_3N_3O_7$.
A suitable commercially available reagent.

Trinitrophenol, ethanolic, TS.
Procedure. Dissolve 1 g of trinitrophenol R in 100 mL of ethanol (~750 g/l) TS.

Tropaeolin O R. C.I. 14270; E103: resorcin yellow; chrysoin S; sulpho orange; acid orange 6; $C_{12}H_9N_2NaO_5S$.
Description. Produces a yellow color in moderately alkaline solutions and an orange color in strongly alkaline solutions (pH range 11.0-12.7).
A suitable commercially available reagent.

Vanillin solution, 2.5%
Dissolve 0.25 g vanillin in 10mL ethanol

Vanillin-sulfuric acid solution
Prepare an ice bath and submerge container with 2.5% vanillin in ethanol
Add 50 mL concentrated sulfuric acid. Mix with cooling while submerged in ice bath. For the phytochemical testing, after spraying with reagent, heat the sprayed TLC plates at 120°C until color has intensified

Van-Urk Salkowski reagent
Mix 50 mL hydrochloric acid and 50 mL absolute ethanol then dissolve 1g p-dimethylaminobenzaldehyde in this solution. Store in brown glass bottle at room temperature. This will be stable for several months (Solution A). Mix 500 mL water and 300 mL sulfuric acid then dissolve 2.03 g ferric chloride hexahydrate in this solution. (Solution B). Mix 1 part Solution A and 3 parts Solution B. Label as spray reagent. In testing for indoles, spray the reagent then heat the sprayed TLC plates at 100°C for 5 mins.

Water, carbon-dioxide-free, R.
Water that has been boiled vigorously for a few minutes and protected from the atmosphere during cooling and storage.

Xylene R. C_8H_{10}.
A suitable commercially available reagent.

D-Xylose R. $C_5H_{10}O_5$.
Description. A white, crystalline powder.
Specific optical rotation. Dissolve 1 g in 10 mL of water; [α]D
A suitable commercially available reagent.

Yeast extract, water-soluble, R.
A suitable commercially available reagent.

Zinc R. Zn; granulate, powder, or dust.
A suitable commercially available reagent.

Zinc, AsR, granulated.
Granulated zinc R that complies with the following tests:
Limit of arsenic. Add 10 mL of stannated hydrochloric acid (~ 250 g/l) AsTS to 50 mL of water, and apply the general test for arsenic; use 10 g of granulated zinc R and allow the reaction to continue for 1 hour; no visible stain is produced.
Test for sensitivity. Repeat the test for arsenic with the addition of 0.1 mL of dilute arsenic AsTS; a faint, but distinct yellow stain is produced.

Zinc acetate R. $C_4H_6O_4Zn,2H_2O$.
A suitable commercially available reagent.

Zinc acetate/aluminium chloride TS.
Procedure. Dissolve 200 g of zinc acetate R and 5 g of aluminium chloride R in sufficient water to produce 1000 mL.

Zinc chloride R. $ZnCl_2$.
A suitable commercially available reagent.

Zinc chloride, iodinated, TS.
Procedure. Dissolve 40 g of zinc chloride R and 13 g of potassium iodide R in 21 mL of water. Add 1 g of iodine R and shake for 15 min. Filter if necessary.
Storage. Store protected from light.

► Measurement of Blanks

Blank testing or performing a blank test means to conduct the same procedure under exactly the same conditions and with the same amount of reagents except for the test substance which is omitted. For TLC/HPTLC, the blank lane refers to the lane in the TLC plate that does not contain the test substance.

► Calculation of Results

Results are calculated based on the assay performed on the test sample, usually with reference to the dried or anhydrous substance or any other specified basis. Where dried or anhydrous substance is used as, the determination of the loss on drying, water content or other property is carried out by the method prescribed in the individual monographs. The word "dried substance" or "anhydrous substance," or other descriptor appears in parenthesis after the result. Where the quantitative determination of a residual solvent is conducted, the amount of residual solvent is considered for the calculation of the results of the assay on the substance, including its optical rotation and specific absorbance, if applicable.

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BLEED

General Information

The items discussed in this chapter are for information only and contain no official standard, tests or assays nor other mandatory specifications with respect to any Pharmacopeial article.

ALTERNATIVE METHODS

The aim of the Philippine Herbal Pharmacopeia is also to prescribe alternative methods or requirements to demonstrate cGMP compliance using different analytical procedures and measurements prescribed in Philippine Herbal Pharmacopeia and in different reference pharmacopeias. This will ascertain that the results are valid and acceptable based on the validation or system suitability criteria as discussed in the chapter, *Validation of Pharmacopeial Methods*.

Alternative procedures are those that may be used to measure an attribute when such procedures control the quality of the drug substance or drug product to an extent that is comparable or superior to the official procedure.

Example: for tablets that have been shown not to degrade during manufacture, it may be permissible to use a spectrophotometric procedure for release as opposed to the official procedure, which is chromatographic. However, the chromatographic procedure should still be used to demonstrate compliance with the acceptance criteria during the shelf life of the product (ICH Q6A)

Methods mentioned below are standardized methods used in the evaluation and quality control of medicinal plants and products. These methods are widely used in research laboratories in the Philippines. These are accepted methods in different pharmacopeias and references and may or may not be employed in the medicinal plant manufacturing industry at present time.

(A) CHROMATOGRAPHIC METHODS

The chromatographic profile of a medicinal plant preparation is made up of the chromatographic pattern of the chemical components of pharmacologically active constituents present in the extracts and/or chemical characteristics of the active constituents and the matrix. With the help of chromatographic profiles, the identification of medicinal plants can be accurately done. It is also advantageous to identify possible markers that are characteristic of the specific extract of plant products because these can be used to assess quality of product. An alternative method to TLC/HPTLC described in the *General Methods* section is high-performance/high pressure liquid chromatography (HPLC), gas chromatography (GC) for specific samples, particularly those containing volatile or thermolabile analytes and other hyphenated chromatographic techniques.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

The preparative and analytical HPLC has been widely used for analysis of medicinal plants because of its high separation capacity. It is used to analyze all constituents of herbal products provided that an optimized procedure is developed which involves optimization of mobile phase and stationary phase, along with other chromatographic parameters. There are basically two types of Preparative HPLC: low-pressure HPLC which is typically under 5 bars and high-pressure HPLC with pressure greater than 20 bars.

High performance liquid chromatography is a chromatographic method that is based on the same principle as column chromatography, i.e. separation by adsorption on a solid matrix or partition in different solvent systems. The mobile phase is pumped into the column packed with particles of various sizes as a stationary phase. The column is packed using a high pressure pump. The components to be separated are carried by the mobile phase and separated by differences in its affinity or adsorption to the stationary phase. This method can be used to separate, identify, and determine purity of

components of mixtures.

► Equipment

This liquid chromatographic system requires the following:

- Injection port where the mixture to be separated is injected
- Chromatographic column
- Detector
- Recording device

Samples are injected into the equipment through the injection port and is transported by the mobile phase. The mobile phase is forced through the column using a high-pressure pump. The column temperature is maintained in a column oven. The components of the sample are separated based on their affinities to the stationary phase and the mobile phase. The liquid chromatography employs separation in either normal or reverse phase. The properties of these components are detected by various types of detectors. There are 2 general types of detectors: 1) specific and 2) bulk property detectors (https://www.pharmaguideline.com/2016/01/different-types-of-hplc-detectors.html).

Under specific HPLC detectors are a) Mass spectroscopic, b) UV/VIS, c) Photodiode array (PDA), and d) fluorescence detectors. Bulk property HPLC detectors include a) electrical conductivity, b) refractive index, c) electrochemical and d) light scattering HPLC detectors. There are other uncommonly used detectors such as evaporative light scattering, IR, transport, aerosol-based, chiral and pulsed amperometric detectors. Detectors are very sensitive and can detect mg quantities of components. The intensity of the signal detected is proportional to the concentration of the particular component being analyzed.

The schematics for an HPLC equipment is shown in ***Figure 4.1.***

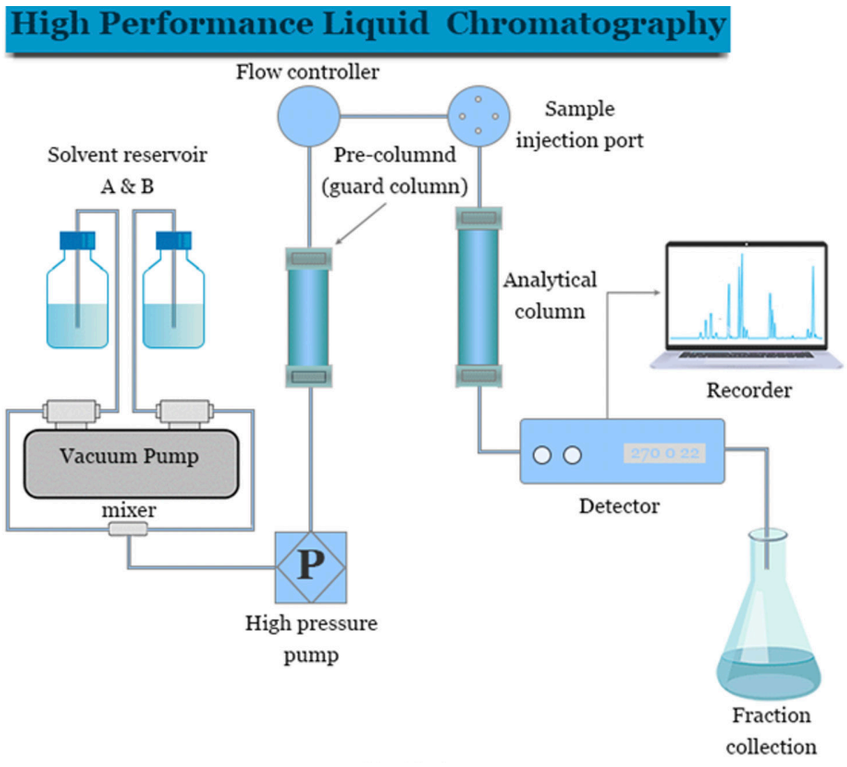

Figure 4.1. *The General Schematic Diagram of an HPLC.*
(Source: https://www.priyamstudycentre.com/2021/12/high-performance-liquid-chromatography-hplc.html)

► **Procedure**

Samples are prepared by extraction in suitable solvents. The extracts are dried and then reconstituted in suitable solvents (DMSO, acetonitrile, etc.). After equilibrating the column, with the prescribed or suitable mobile phase indicated in the monograph, adjusting the flow rate at temperature specified, baseline is taken until a stable profile is obtained. Standards or a quantity of sample, the concentration of which is greater than the LOQ, is injected through the sample valve by using a microsyringe. The separated components are detected by the detector which sends a signal to the recorder to display a chromatogram. The components are distributed as constant ratio k in the mobile and stationary phases where k is the amount of substance in the stationary phase/ amount of substance in the mobile phase. This ratio is also called the capacity factor or mass distribution ratio. The relation of the capacity factor, the time the mobile phase passes through the column, tO, and retention time, tR is determined using the equation below. Retention times of the components being separated remain constant under the same conditions.

$$tR = (1 + k')\, tO$$
$$or\ k' = tRtO - 1$$

- $t0$ = the retention time of a non-retained (k=0) substance
- tR = the retention time of a test specimen

The relative retention times are used to identify the components of the sample in relation to the retention time of standards applied to the column and run under the same conditions. The distance between peaks is the resolution, R of the components and is also called the separation efficiency. The chromatographic profile should show sharp peaks, avoiding tailing as much as possible.

System suitability. The chromatographic parameters must be specified in the individual monographs to ensure the effectiveness and suitability of the method. Data (column efficiency, precision, tailing factor, resolution, retention time, calibration curve, peak area, and recovery) for replicate injections of standard or test solutions will ensure reproducibility and accuracy of the analytical method and they need to be specified in the monographs. Reproducibility of repeat injections is described as relative standard deviation of the runs and determined by calculating RSD of peak area from specified injection.

GAS CHROMATOGRAPHY (GC)

It is used for authentication and quality control of volatile samples. The high selectivity of capillary columns enables separation of many volatile compounds simultaneously within comparatively short times. The disadvantage of GC is that this method is not convenient for the analysis of samples which are thermolabile and non-volatile. Derivatization procedures may be necessary to render analytes volatile for GC analysis. But the process of derivatization has to undergo optimization and validation procedures.

HYPHENATED TECHNIQUES

These are chromatographic separation techniques which are coupled to various detection methods such as mass spectrometry (MS), nuclear magnetic resonance (NMR), infrared spectroscopy (IR), etc. These techniques provide information about the structure of the compound present in the chromatogram and provide higher sensitivity. Various hyphenated techniques that are used include:

- **Liquid chromatography-mass spectrometry (LC-MS)**

  This technique is used to characterize a wide variety of plant constituents, ranging from small molecules to macromolecules such as peptides, proteins, carbohydrates and nucleic acids. Recent advances in detection include electrospray, thermospray, and ion spray ionization techniques which offer unique advantages of high detection, sensitivity and specificity. Isotope patterns can be detected by this technique.

- **Liquid chromatography-nuclear magnetic resonance (LC-NMR)**

  The combination of chromatographic separation technique with NMR spectroscopy is one of the most useful and time-saving methods for the separation and structural elucidation of unknown compounds, mainly for the structural elucidation of light- and oxygen-sensitive substances.

- **Gas-chromatography-mass spectrometry (GC-MS)**

  It is used for the analysis of thermolabile constituents of herbal medicines, especially because the hyphenation with MS provides reliable information for the qualitative analysis of the constituents. The flow rate from the capillary column is low so that the column output can be fed directly into the ionization chamber of MS. The mass detector in GC is the Ion Trap Detector (ITD). In this instrument, ions are created from the eluted sample by chemical ionization and stored in a radio frequency field. The trapped ions are then ejected from the storage area to an electron multiplier detector.

(B) DNA FINGERPRINTING

In spite of new advances developed in modern medicines, medicinal plant formulations are still extensively used as therapeutic agents for treatment of several diseases. Herbal drug technology is used for converting plant materials into medicines and it requires a standardization process that will evaluate the crude herbal extracts' quality and purity. This standardization process includes various parameters like morphological, microscopical, physical, chemical and biological characterization. In addition to the mentioned processes, there is an increasing interest in including DNA fingerprinting in establishing a quality control process for medicinal plants and plant products due to its accuracy and reliability. DNA fingerprinting utilizes the intricate characteristic of the deoxynucleotide acid (DNA) molecule, that is, the specific sequences of the DNA nitrogenous bases (adenine, cytosine, guanine, and thymine). This technique has been used in botanical studies such as the conservation and protection of biodiversity, identification of genetic markers for trait identification, and the determination of gene diversity and variation.

DNA fingerprinting is defined as the application of genetic/molecular marker techniques to identify species and differentiate one cultivar from another cultivar. Specific techniques like restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD), simple sequence repeat (SSR) sequencing of rDNA-ITS region and DNA barcoding are all methods of DNA fingerprinting whose principles and mechanisms depend on the sequence of the DNA. The development of the DNA fingerprints or "barcodes" require the use of DNA markers for the proper identification and standardization of plant species. In the development of the pharmacopeia, the DNA barcoding, also known as DNA fingerprinting, was used for the molecular identification of the medicinal plants used for drug formulation. The DNA fingerprint (or DNA barcodes) are the barcode-like patterns that can be used to determine the uniqueness of an individual from the other species which are produced. This DNA marker-based analysis has been proven useful in medicinal plant standardization.

DNA/GENETIC MARKERS

These are genes or DNA sequences with known locations on a chromosome and associated with a particular gene or trait. It can be described as a variation, which may arise due to mutation or alteration in the genomic. A genetic marker may be a short DNA sequence, such as a sequence surrounding a single base-pair change (single nucleotide polymorphism SNP). Some commonly used types of genetic markers are RFLP, AFLP, RAPD, VNTR (or Variable number tandem repeat), Microsatellite polymorphism, SNP, STR (or Short tandem repeat), and SFP (or Single feature polymorphism).

METHODOLOGY

The basic methodology of DNA profiling used for the medicinal plants included in the pharmacopeia involves DNA isolation, isolated DNA quantification and purity determination, amplification of the DNA through polymerase chain reaction, amplicon sequencing, isolation of DNA from plant cells, quantification and quality assessment of isolation.

1. **DNA Isolation**

   Fresh leaves from the herbal plant are sampled and then brought to the laboratory for genomic DNA isolation. DNA from plant tissue is isolated by mechanically destroying the cell wall, cell membrane, and the nuclear membrane through the CTAB isolation method or by using DNA extraction kits. These procedures liberate the DNA as well as the cell components into the solution. The DNA isolated is obtained from the young fresh leaves of the medicinal plants.

2. **DNA Quantification and Quality Assessment**

   The concentration and purity of the genomic DNA isolated are quantified and assessed using UV-VIS spectrophotometry which shows the A260/A280. The DNA isolates with a ratio between 1.70 to 1.90 showed the highest purity and is considered for the succeeding downstream processes.

3. **DNA Marker Synthesis**

   Specific DNA regions are amplified using DNA markers (forward and reverse primer pairs). Several DNA markers, particularly rbcL (ribulose bisphosphate carboxylase large subunit gene) and matK (maturase K gene), are synthesized, and from these markers, the most appropriate markers are selected and then optimized. In the optimization process, the selected DNA markers are tested against the DNA isolates and then the most appropriate cycling parameters and temperatures for the denaturation, annealing, elongation, and final elongation are identified.

4. **Polymerase Chain Reaction (PCR)**

   This is an in vitro method used to amplify specific DNA segments by a three-step process that is carried out in repeated cycles. A PCR cocktail is prepared following the PCR kit manufacturer's instructions and then the isolated DNA is added. The cocktails with the DNA are placed in a thermal cycler. The cycler is programmed for the three-step process. The amplification starts with the denaturation process to expose the DNA bases, followed by the annealing of the DNA markers to its complementary DNA bases. Elongation or primer extension using Taq polymerase follows wherein amplicons of the specific DNA sequence are produced. The final extension elongates the amplicons and ensures full-length polymerization of the target DNA sequence.

5. **DNA Sequencing and Sequence Analysis**

   The produced amplicons are sent to a DNA sequencing facility. The method of choice for the sequencing is a capillary sequencing. The obtained DNA sequences are identified by comparing with the available sequences in the databases. Moreover, further data analysis is performed using bioinformatics software. Basic Local Alignment Search Tool (BLAST) analysis from Genbank and Identification Engine of Barcode of Life (BOLD) Systems are used to identify plant species based on sequence alignment. The species identification with the highest similarity index (may be 99 or 100%) is used as the identity of the sample. With any or all of the markers used, this has to be consistent with the species with the closest match.

(C) BIOAUTOGRAPHY

Planar chromatographic analysis like thin-layer chromatography is an effective and inexpensive technique for the phytochemical analysis of plant extracts to identify bioactive compounds. TLC bioautography is a hyphenated technique combining planar chromatographic separation and in situ biological activity detection using various methods based on the characteristics of active constituents. This coupled method has been receiving much attention in screening bioactive natural products because of its properties of being simple, rapid, inexpensive, and effective.

It can be performed both in highly developed laboratories as well as in small research laboratories which have minimum access to sophisticated equipment. Bioautography offers a simple, rapid and inexpensive method for the chemical and biological screening of complex medicinal plant extracts, with subsequent bioassay-guided isolation. In 1946, Goodall and Levi introduced paper chromatography (PC)-based bioautography for the first time to estimate the purity of penicillin. In 1961, Fisher and Lautner and Nicolaus et al introduced thin-layer chromatography (TLC)-based bioautography.

Generally, planar chromatographic techniques, which includes TLC and paper chromatography are used for bioautography, but the detection method can be successfully improved by the application of advanced chromatographic tools, namely, a high performance thin-layer chromatography (HPTLC), and planar electrochromatography (PLC). The major applications of bioautography are the rapid screening of a large number of samples for bioactivity, namely, antibacterial, antifungal, antioxidant, enzyme inhibition, etc. and in the target-directed isolation of active compounds.

For determining antimicrobial activity, three (3) general bioautographic assays have been developed. These include a) direct bioautography where the microorganisms grow directly on the TLC plates, contact bioautography, where the antimicrobial compounds are transferred from the TLC plate to an inoculated agar plate through direct contact, and agar overlay or immersion bioautography; where a seeded agar medium is applied onto the TLC plate.https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3005382/

Inhibition of enzyme activity can be determined by enzymatic bioautography which enables detection of the inhibitors, including some natural products, adsorbed on the TLC plates. (Methods Mol Biol. 2020;2089:179-189. doi: 10.1007/978-1-0716-0163-1_12)

BIOLOGICAL METHODS

Biological methods are described for the assay of certain substances and preparations whose potency cannot be adequately assured by chemical and physical analysis. The principle applied whenever possible throughout these assays is that of comparison with a standard preparation so as to determine how much of the substance to be examined produces the same biological effect as a given quantity, the Unit, of the standard preparation. It is an essential condition for such methods of biological assay that the tests on the standard preparation and on the substance to be examined be carried out at the same time and under identical conditions. The standard could be a material established as such by an international organization (eg USP, WHO) or it could be an internal standard.

References

Eu Phar 8th ed.
USP 1032

PLANT PHOTOGRAPHS

Many medicinal plants look alike and for this reason, one photo of the entire plant is not enough to make a definitive botanical identification. Examples are *Blumea balsamifera* (sambong) and *Vitex negundo* (lagundi), which looks like *Blumea lacera* (also called sambong in Mindanao) and *Vitex trifolia* (lagunding dagat), respectively. Other commonly misidentified medicinal plants are *Curcuma aromatica* for *Curcuma longa* (Luyang Dilaw), *Hydrocotyle vulgaris* for *Centella asiatica* (Takip Kuhol), and *Tridax procumbens* for *Bidens pilosa* (Dadayem). To ensure proper identification, it is important to document different plant parts. Here are some important guidelines in taking plant photographs.

1. Take photographs of the entire plant, showing scale if possible by including a recognizable object in the photo, such as a ruler or pencil, or a person standing next to it.

2. Take a picture of a stem to show if the attachment of the leaves are alternating or opposite.

3. Take close-ups of the leaves, both its upper and lower surfaces, making sure that both ends of the leaf are in the photo.

4. Take close-ups of the flowers (front and back) and fruit, if present.

5. Take a photo of the habitat where the plant is collected.

Photograph any feature of the plant that might make it easier to identify it later, for example stipule, leaf bud, spines, and hairs. Including all representative parts of the plant makes it more likely that the species can be determined without having a specimen-in-the-hand. A detailed guide for photodocumentation can be found at:

https://www.canterbury.ac.nz/media/documents/oexp-science/biological-science/Which-parts-of-the-plant-should-I-photograph_Matt-Walters.pdf

The following information must be provided on the photographs: Date photo is taken, Name of photographer, Location and general GPS coordinates, and Altitude.

PROCEDURE IN MAKING PERMANENT SLIDES OF MICROSCOPIC SECTIONS OF PLANT VEGETATIVE ORGANS

In making plant sections into permanent slides for microscopic studies, several steps are undertaken depending on the method being adopted.

Selected plant parts are cut into very thin sections either through freehand sectioning or with the use of a microtome.

1. FREEHAND SECTIONING

Cut the plant part (vegetative or reproductive part) chosen for the study (in this case a leaf for this present research). After cutting the desired leaf, trim the leaf further, making sure the midrib with the leaf blade, is included in this leaf section. The size of the leaf segment including the midrib, has to fit into two small slices of a green papaya fruit mesocarp. The leaf fragment somehow is sandwiched between these two slices of papaya mesocarp. To strengthen this set-up, a thread (used for sewing) is placed around it repeatedly. Once tied securely, place the prepared plant fragment with the papaya mesocarp in a clean container with water to allow rehydration of the cut specimen. After 30 min or so, make thin sections of the leaf using a new razor blade. The cut sections of the leaf are separated from the papaya supporting fruit slices and allow these leaf tissue thin sections to be placed in a small container with water. The cut sections are allowed to stand in a water bath to prevent the wrinkling of the cells/tissues of the cut tissue sections. View these sections using a compound microscope, to eliminate freehand sections that are not one cell thick. The selected good sections are then fixed/killed in a small syracuse disc (or improvised previously cut small bottle bottom) filled with 70% ethyl alcohol for at least an hour or so depending on the texture of the leaf. After fixing the sections, these are allowed to run thru a staining dehydration series.

Staining with safranin and counterstaining with fast green may take 24 hours or less depending on the consistency of the plant specimen. This is done initially by soaking the sections in safranin solution. Once the safranin has been absorbed by the tissues, the sections are removed and rinsed to remove excess stain. Fast green is used as the counter stain. Safranin are attracted to thick-walled cells while fast green stain adheres well to thin-walled cells.

As the sections are intended to last for a long time, the tissues in these sections have to be preserved. This is done by replacing the water content by running the sections in an alcohol dehydration series. Thus, after fixing and staining, the sections are subjected to an ethyl alcohol dehydration series starting with 70%, 85%, 95%, 100% ethyl alcohol. Solvent

used in this alcohol series (of 70%, 85% and 95% ethyl alcohol) is distilled water. After dehydration, the sections are subjected to a clearing treatment using 25%,50%,75%,100% xylene series (with 100% ethyl alcohol as the other solvent to come up with the 25%, 50% and 75% xylene solution). Once the sections are cleared, a clean slide is prepared for each section. In each slide, place a drop of Canada balsam before placing singly a section in each slide. Cover with a clean cover slip and allow to dry overnight in an oven. Once completely dried, clean the sides of the cover slip with the mounted section using a soft clean lint-free rag, moistened with a small amount of xylene to remove excess Canada balsam flowing out from the cover slip. Label properly by placing adhesive labeling tag on the side of the slide.

2. MICRO SECTIONING USING A MICROTOME

A microtome is a mechanical gadget/equipment used to cut thin sections of plant or animal tissues with the use of a sharp blade or knife. There are several types of microtomes:

A. Hand microtome

The hand microtome is the simplest microtome that can cut thin sections of plant or animal materials for microscopic studies. It cuts these materials held in place using this gadget by hand in following a set calibration in the gadget itself.

Using a hand microtome is similar to the procedure in making micro-sections by freehand method. Only this time, instead of using green papaya fruit as support in making micro-sections of a plant part, a hand microtome is used. Sectioning of the cut plant material in this case is made possible with a small knife where section of cut plant is usually less than 10mm in diameter and 25 to 35 mm in length.

B. Sliding Microtome

The sliding microtome is an instrument that allows a plant or animal material that is held in place by a specimen holder, with the latter remaining stationary while the attached microtome blade runs obliquely across the mounted plant or animal material. (https://www.merriam-webster.com/medical/sliding%20microtome)

For every run of the knife to the fixed specimen holder bearing the tissue, the section can be retrieved using a moistened camel's brush. Each section is then placed in a bowl of water to prevent wrinkling of the tissue due to water loss within the cells. Screening for good sections are made using a compound microscope. The good sections are then fixed/killed by placing these in a fixing agent like 70% ethyl alcohol. These sections may be fixed for an hour to 3 hours depending on the specimen consistencies. Sections are then soaked in safranin solution for 4 hours or more, depending on the specimen. There are specimens that require staining overnight though. Excess stain can be removed by allowing and to pass through slow running water. If the stain is alcohol-based, destain sections using 70% ethyl alcohol for a short time. When the initial stain is in place, allow the sections to be soaked in fast green as a counter stain. After the preferred staining has been achieved, the dehydration-clearing process follows. The freehand sectioning procedure for the alcohol-based dehydration series and xylene based-clearing series and mounting of sections on clean slides can be adopted.

C. Rotary Microtome

Similar to the sliding microtome, the rotary microtome is an instrument that also cuts specimens to form sections for microscospic studies. Only in this case the specimen embedded in a paraffin block that is mounted in the specimen holder of the microtome moves towards the stationary blade. As the paraffin block with the specimen moves towards the knife according to the thinness set, several thin sections form into a paraffin ribbon made of several serial sections. Thus, in making sections using a rotary microtome, a paraffin block with the plant specimen embedded in it has to be prepared following basic fixing/killing dehydration series. For the plant sections, a rotary microtome is used to make prepared slides of specimens for this research. The following steps are made before cutting the specimen using the microtome to wit.

► Fixing/Killing of the Cut Plant Part (i.e. leaf)

Specimen is fixed in 70% ethyl alcohol for 24 hours or more.

► Dehydration-Clearing Series

After fixing, the plant part is subjected to a dehydration-clearing series as follows:

- 95% ethyl alcohol
- Isopropyl alcohol
- Iso-xylene
- Xylene (until cleared)

► Embedding Paraffin of Plant Part

Once specimen has been cleared after going thru the xylene treatment, paraffin embedding of specimen commences following the series:

- Xylene-Paraffin
- Paraffinization: 30-minute interval (inside the embedding chamber)
- Soft paraffin 1
- Soft paraffin 2
- Soft paraffin 3
- Hard paraffin 1
- Hard paraffin 2
- Hard paraffin 3
- Final Paraffin (embedded in a paraffin block)

► Sectioning (Using A Rotary Microtome) and Affixing Sections on the Slide

Trim the paraffin block with specimen according to the size of the specimen holder. Put in place the paraffin block on the specimen holder as the paraffin block with specimen passes through the knife, ribbons of overlapping paraffin sections are formed.

► Affixing Sections on the Slides

These paraffin ribbons are placed initially in cold water then in warm water before affixing. These sections are placed on the slides treated previously with glycerin as adhesive or Mayer's adhesives so the sections on these ribbon are fixed on the slide.

► Staining of Slides with Specimens Adhered to the Slides Using Safranin Counterstained Fastgreen Following the Staining-Dehydration-Cleaning Procedure

- Xylene (3x)
- Isopropyl (3x)
- 95% ETOH
- 70% ETOH
- 50% ETOH
- Distilled water
- Safranin (for 1-2 min)
- 95% ETOH
- Fastgreen (for 10 dips)

- 95% ETOH
- Isopropyl (3x)
- Iso-Xylene
- Xylene (3x)

► **Mounting of Coverslip on the Stained Sections Affixed on the Slide**

- Dry the area on the slide surrounding the sections
- Place Canada balsam around the sections
- Carefully place a clean cover slip over the area where the Canada balsam is placed
- Place the slide with cover slip in an oven overnight
- Clean excess balsam seeping out of the cover slip using a small piece of rag wet with very small amount of xylene

► **Label the Prepared Slides Properly**

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Part I. Herbal Medicines

BLUMEAE BALSAMIFERAE

Blumeae Balsamiferae Folium
Sambong Leaf

DEFINITION

Blumeae Balsamiferae Folium is the dried leaf of *Blumea balsamifera* (L.) DC. (Fam. Compositae/ Asteraceae).

SCIENTIFIC NAME

Blumea balsamifera (L.) DC.

SYNONYMS

Baccharis balsamifera Stokes
Baccharis gratissima Blume ex DC.
Baccharis salvia Lour.
Blumea balsamifera var. *balsamifera*
Blumea balsamifera var. *microcephala Kitam*

SELECTED COMMON NAMES

alibhon (Pal., Ant., Neg. Occ., Sav., Sur., Zambo., S. Cot., S. Kud.), *alibum*, *alimon* (P. Bis.), *alimhon* (Cap.), *ayoban*, *gabuen*, *gitin-gitin*, *hamlibon*, *kambibon*, *lakdanbulan*, *lalakdan* (Bis.), *bukadkad* (S.L. Bis.), *dalapot* (C. Bis.), *gabon* (Mis., Cam., Dav.), *hanalibon* (Akl.), *kaliban* (Tag.), *kalibutan* (Tagb.), *labulan* (Sub.), *lakadbulan* (Bis., Sul.), *libon* (S. Cot.), *pahid* (Sur.), *sambun* (Sul.), *sob-sob*, *subusub* (Ilk.), *takamain* (Bag.); *blumea camphor*, *ngai camphor* (Eng.), *bai mat* (Cam.); *phi ma 'sen*, *nat* (Lao PDR); *capa*, *sembung*, *sembung utan*, *sembung gantung*, *sembung gula*, *sembung kuwuk*, *sembung lego* (Ind.); *chapa*, *chapur*, *sembong*, *capa*, *telinga kerbau* (Mal.), *poungma-theing* (Mya.); *naat*, *nat yai, naat yai*, *kamphong*, *akm phung*, *dai*, *dai bi* (Thai.); *c[aa]y t[uwf]bi-*, *cay dai be* (Viet.)

MAJOR CHEMICAL CONSTITUENTS

Blumeae Balsamiferae Folium yields a volatile oil (0.1 to 0.4%) containing chiefly(-)-borneol, 25% (-)-camphor, and 75% limonene. The leaf contains palmitic and myristic acids.

MORPHOLOGICAL DESCRIPTION OF THE PLANT

This strongly aromatic, perennial herb or half woody shrub can reach a height of 1-4 m and has a diameter of the stem at 2.5 cm. The internodes in the upper portion of the stem have nonglandular hairs. It has simple petiolated leaves. The peduncled head of leafy panicled florets is surrounded by green, narrow, hairy involucral bracts. Flowers are yellow and discoidal, composed of numerous, tiny peripheral ones with tubular corolla and a few large central flowers with campanulate corolla. The achene fruit is with tuft of small hairs.

MACROSCOPIC DESCRIPTION

The simple, petiolated leaf has an elliptic to oblanceolate outline, with serrated margins and acute/obtuse base narrowing down to the short petiole. It has a hairy underside as well.

MICROSCOPIC DESCRIPTION

Transverse section of the leaf of *sambong* shows the midrib is convex on the lower surface. This has only one layer of epidermal cells that are almost circular. Numerous unicellular and tapering trichomes are present. However, trichomes in the upper epidermis are less dense than in the lower epidermis. Five to seven layers of collenchyma lie below the upper epidermis and above the lower epidermis. The main vascular bundle is centrally located in the predominantly parenchymatous midrib. Smaller parenchyma cells are found nearer to the peripheral side of the vascular bundle. Cells are tightly packed in the mesophyll layer (composed of palisade and spongy cells). Anomocytic stomata (no subsidiary cells surrounding the guard cell area) are found in both the lower and upper layers of the waxy epidermis.

WARNING

Sambong can cause allergic reactions for people sensitive to ragweed plants and its relatives. Side effects may include itching and skin irritation.

PACKAGING AND STORAGE

Preserve in well-closed, light-resistant containers.

LABELING

Print the official title and the national title of the herbal material on the label as directed under *General Notices and Requirements* Part I.

TRADITIONAL USES

It is used for wounds and cuts, rheumatism, anti-diarrhea, anti-spasm, colds, and cough. Also used as antidiuretic, maintain urinary tract health, and remove excess uric acid. Has antibacterial and antifungal properties. It is used as anticancer, antioxidant, antimicrobial, antiinflammation, antiplasmodial, platelet aggregation, enhancing percutaneous penetration, wound healing, anti-obesity, and insecticidal. Also used for respiratory tract disorders (e.g. sinusitis, asthmatic bronchitis, influenza).

Blumeae Balsamiferae Folium Pulveratum
Sambong Leaf Powder

DEFINITION

Blumeae Balsamiferae Folium reduced to powder form.

SCIENTIFIC NAME

Blumea balsamifera (L.) DC.

SYNONYMS

Baccharis balsamifera Stokes
Baccharis gratissima Blume ex DC.
Baccharis salvia Lour.
Blumea balsamifera var. *balsamifera*
Blumea balsamifera var. *microcephala Kitam*

SELECTED COMMON NAMES

alibhon (Pal., Ant., Neg. Occ., Sav., Sur., Zambo., S. Cot., S. Kud.), *alibum*, *alimon* (P. Bis.), *alimhon* (Cap.), *ayoban*, *gabuen*, *gitin-gitin*, *hamlibon*, *kambibon*, *lakdanbulan*, *lalakdan* (Bis.), *bukadkad* (S.L. Bis.), *dalapot* (C. Bis.), *gabon* (Mis., Cam., Dav.), *hanalibon* (Akl.), *kaliban* (Tag.), *kalibutan* (Tagb.), *labulan* (Sub.), *lakadbulan* (Bis., Sul.), *libon* (S. Cot.), *pahid* (Sur.), *sambun* (Sul.), *sob-sob*, *subusub* (Ilk.), *takamain* (Bag.); *blumea camphor*, *ngai camphor* (Eng.), *bai mat* (Cam.); *phi ma 'sen*, *nat* (Lao PDR); *capa*, *sembung*, *sembung utan*, *sembung gantung*, *sembung gula*, *sembung kuwuk*, *sembung lego* (Ind.); *chapa*, *chapur*, *sembong*, *capa*, *telinga kerbau* (Mal.), *poungma-theing* (Mya.); *naat*, *nat yai*, *naat yai*, *kamphong*, *akm phung*, *dai*, *dai bi* (Thai.); *c[aa]y t[uwf]bi-*, *cay dai be* (Viet.)

MAJOR CHEMICAL CONSTITUENTS

Blumeae Balsamiferae Folium yields a volatile oil (0.1 to 0.4%) containing chiefly()-borneol, 25% ()-camphor, and 75% limonene. The leaf contains palmitic and myristic acids.

WARNING

Sambong can cause allergic reactions for people sensitive to ragweed plants and its relatives. Side effects may include itching and skin irritation.

PACKAGING AND STORAGE

Preserve in well-closed, light-resistant containers.

LABELING

Print the official title and the national title of the herbal material on the label as directed under *General Notices and Requirements* Part I.

IDENTIFICATION

a. Standard Solution

1 mg/mL of Eriodictyol RS in ethanol. Sonicate for 1 min and mix by vortex.

b. Sample Solution

Sonicate 5 g of *Blumea balsamifera* Leaf, finely powdered, in 25 mL ethanol for 30 min. Filter the mixture using filter paper and use the collected filtrate.

c. Chromatographic System

i. **Adsorbent:** TLC plate silica gel 60 F254 (20x10 cm)

ii. **Application volume:** 0.2 µL sprayed as 9 mm bands using suitable device

iii. **Relative humidity:** Condition the plate to a relative humidity of about 33% for 15 min using a suitable device.

iv. **Developing solvent system:** a mixture of chloroform and methanol (8:1)

v. **Developing distance:** 85 mm

vi. **Derivatization reagent:** 1% aluminum chloride in methanol

d. Analysis

i. **Samples:** *Standard Solution and Sample Solution* Apply the samples to the TLC plate and pre-dry. Develop the chromatogram in a chamber saturated for 20 min using a suitable device and remove the developed plate after drying. Examine under normal light, short wavelength UV light (254 nm), and long wavelength UV light (366 nm). Treat with derivatization reagent, and dry at room temperature for 10 mins. Examine again under normal light, short wavelength UV light (254 nm), and long wavelength UV light (366 nm).

ii. **Acceptance criteria:** The sequence of zones present in the chromatogram of the sample solution must exhibit similar pattern to the reference chromatogram. Other faint bands may be present in the chromatogram of the sample solution. See *Annex* for the reference chromatogram.

FOREIGN MATTER

<2> not more than 2%

LOSS ON DRYING

<3> not more than 10%

ALCOHOL (95% v/v)–SOLUBLE EXTRACTIVES

<4> not less than 8%

WATER-SOLUBLE EXTRACTIVES

<4> not less than 10%

TOTAL ASH

<5> not more than 13%

ACID-INSOLUBLE ASH

<5> not more than 5%

WATER-SOLUBLE ASH

<5> not more than 7%

TEST FOR TOXIC HEAVY METALS

<54> The toxic heavy metal limits should conform to the specified limits stated in AO 184 s. 2004. Sample should not have more than 0.3 ug/g (ppm) for arsenic and cadmium, 10 ug/g (ppm) for lead, and 0.5 ug/g (ppm) for mercury.

AFLATOXIN

<57> Total aflatoxin shall conform to the prescribed specification of 20 mcg/kg and aflatoxin B1 of 10 mcg/kg by ELISA method or liquid chromatography

PESTICIDE LIMITS

<56> shall conform to the limits defined by WHO/FAO Codex Alimentarius

MICROBIAL LIMIT

<52> The total aerobic microbial count does not exceed 10^5 cfu/g, the total combined molds and yeast count does not exceed 10^3 cfu/g, and it meets the requirements of the tests for the absence of *Salmonella* species and *Escherichia coli.* Other enterobacteria do not exceed 10^3 cfu/g.

TRADITIONAL USES

It is used for wounds and cuts, rheumatism, anti-diarrhea, anti-spasm, colds, and cough. Also used as antidiuretic, maintain urinary tract health, and remove excess uric acid. Has antibacterial and antifungal properties. It is used as anticancer, antioxidant, antimicrobial, antiinflammation, antiplasmodial, platelet aggregation, enhancing percutaneous penetration, wound healing, anti-obesity, and insecticidal. Also used for respiratory tract disorders (e.g. sinusitis, asthmatic bronchitis, influenza).

Blumeae Balsamiferae Tablet
Sambong Tablet

DEFINITION

Blumeae Balsamiferae Folium Pulveratum processed into tablet form. Each tablet of Blumeae balsamiferae may contain 250 or 500 mg of dried *sambong* leaf powder.

WARNING

Side effects may include itching and skin irritation. Avoid use in patients with signs of renal blockage. For patients with excretory urogram showing signs of renal obstruction, consultation with a physician is necessary. Safety in pregnant and lactating women has not been established.

PACKAGING AND STORAGE

Store at a temperature not exceeding 30˚C.

LABELING

Print the official title and the national title of the herbal material on the label as directed under *General Notices and Requirements* Part I.

UNIFORMITY OF DOSAGE UNITS

<31> meets the requirements.

DISINTEGRATION

<38> All of the tablets disintegrated completely with no palpable firm core in not more than 30 min.

FRIABILITY

<37> maximum mean weight loss from the samples is not more than 1%

MICROBIAL LIMIT

<52> The total aerobic microbial count does not exceed 10^5 cfu/g, the total combined molds and yeast count does not exceed 10^3 cfu/g, and it meets the requirements of the tests for the absence of *Salmonella* species and *Escherichia coli*. Other enterobacteria do not exceed 10^3 cfu/g.

MEDICAL USES

Diuretic, Antiurolithiasis agent, Antihypuricemic agent

EHRETIAE MICROPHYLLA

Ehretiae Microphylla Folium
Tsaang Gubat Leaf

DEFINITION

Ehretiae Microphylla Folium is the dried leaf of *Ehretia microphylla* Lam. (Fam. Boraginaceae).

SCIENTIFIC NAME

Ehretia microphylla Lam.

SYNONYMS

Carmona heterophylla Cav.
Carmona microphylla (Lam.) G.Don
Carmona retusa (Vahl) Masam.
Cordia coromandeliana Retz. ex A.DC.
Cordia retusa Vahl
Ehretia heterophylla Spreng.

SELECTED COMMON NAMES

tsaang gubat, *cha*, *chaang-bundok*, *talibunog*, *kalimumoog*, *kalamoga*, *kalimong* (Tag.); *alangitngit*, *mangit* (Tag., Bis.), *putputai* (Bik.), *alangit* (Bis.); *balingsua* (C. Bis.); *buntaitai*, *mara-mara* (P. Bis.); *buyo-buyo*, *buyok-buyok*, *santing* (Sul.); *gari* (Bag.); *icha*, *icha-nga*, *atap*, *icha-ti-bakir*, *itsa* (Ilk.); *kalimumug* (Mag); *mara-mara* (bik., SL. Bis.); *maratia* (ibn); *palupo* (Iv.); *putputai* (Bik.), *Philippine tea tree*, *Fukien tea tree*, *scorpionbush*, *wild tea* (Eng.); *kinangan*, *serut lanang*, *pinaan* (ind.); *khoi cheen*, *chaa yeepun*, *chaa* (Thai.); *kim li[ee]n*, *c[uf]m r[uj]n, b[uf]m r[uj]n* (Viet.)

MAJOR CHEMICAL CONSTITUENTS

Ehretiae Microphylla Folium contains the triterpenes α- and β-amyrin, and baurenol.

MORPHOLOGICAL DESCRIPTION OF THE PLANT

Tsaang gubat is a medium-sized shrub to a small tree, measuring 1-3 m high with green obovate petiolated leaves. It bears small white axillary flowers borne in clusters, with lanceolate sepals forming four to five somewhat hairy, green calyx lobes. Fruit is a yellow, globose drupe (fleshy on the outer part and stony inside), which is red to brownish orange, smooth and glossy when ripe. Only one to five seeds are found in a single fruit.

MACROSCOPIC DESCRIPTION

The petiolated leaves are in clusters of three to five, toothed margin towards the apex. The leaf tip is shallowly indented (retuse).

MICROSCOPIC DESCRIPTION

Large upper epidermal cells with stomata characterize the outermost layer of cells of the leaf blade of tsaang gubat. Some are warty covered by trichomes. The mesophyll is made up of elongated chlorenchymatous cells in the upper palisade layer and irregularly shaped spongy parenchyma with large intercellular spaces, lying horizontal to the lower epidermis. Prismatic cells are present in both mesophyll layers. Midrib is broadly convex below with a central vascular bundle where lignified xylem (upper portion)and phloem (lower portion) are surrounded by large waxy walled parenchyma cells with aggregate prismatic crystals. Collenchyma cells can be found below the upper epidermis and above the lower epidermis in the midrib region.

WARNING

May cause allergic reactions when in contact with the skin.

PACKAGING AND STORAGE

Preserve in well-closed, light-resistant containers.

LABELING

Print the official title and the national title of the herbal material on the label as directed under *General Notices and Requirements* Part I.

TRADITIONAL USES

Traditionally used to treat stomachache, abdominal pain, diarrhea, preventing tooth decay, and disinfectant wash during childbirth. Also used as antinociceptive, antibacterial, analgesic, hepatoprotection, enhanced wound healing, prevent tooth decay, treatment of cough and skin disease, and antimutagenic.

Ehretiae Microphylla Folium Pulveratum
Tsaang Gubat Leaf Powder

DEFINITION

Ehretiae Microphylla Folium reduced to powder form.

SCIENTIFIC NAME

Ehretia microphylla Lam.

SYNONYMS

Carmona heterophylla Cav.
Carmona microphylla (Lam.) G.Don
Carmona retusa (Vahl) Masam.
Cordia coromandeliana Retz. ex A.DC.
Cordia retusa Vahl
Ehretia heterophylla Spreng.

SELECTED COMMON NAMES

tsaang gubat, *cha*, *chaang-bundok*, *talibunog*, *kalimumoog*, *kalamoga*, *kalimong* (Tag.); *alangitngit*, *mangit* (Tag., Bis.), *putputai* (Bik.), *alangit* (Bis.); *balingsua* (C. Bis.); *buntaitai*, *mara-mara* (P. Bis.); *buyo-buyo*, *buyok-buyok*, *santing* (Sul.); *gari* (Bag.); *icha*, *icha-nga*, *atap*, *icha-ti-bakir*, *itsa* (Ilk.); *kalimumug* (Mag); *mara-mara* (bik., SL. Bis.); *maratia* (ibn); *palupo* (Iv.); *putputai* (Bik.), *Philippine tea tree*, *Fukien tea tree*, *scorpionbush*, *wild tea* (Eng.); *kinangan*, *serut lanang*, *pinaan* (ind.); *khoi cheen*, *chaa yeepun*, *chaa* (Thai.); *kim li[ee]n*, *c[uf]m r[uj]n, b[uf]m r[uj]n* (Viet.)

MAJOR CHEMICAL CONSTITUENTS

Ehretiae Microphylla Folium contains the triterpenes α- and β-amyrin, and baurenol.

WARNING

May cause allergic reactions when in contact with the skin.

PACKAGING AND STORAGE

Preserve in well-closed, light-resistant containers.

LABELING

Print the official title and the national title of the herbal material on the label as directed under *General Notices and Requirements* Part I.

IDENTIFICATION

a. Standard Solution

(1) 2.0 mg/mL of Caffeic Acid RS in methanol and (2) 2.0 mg/mL of Rosmarinic acid in methanol. For each solution, sonicate for 1 min and mix by vortex.

b. Sample Solution

Sonicate 2.5 g of *Ehretia microphylla* Leaf, finely powdered, in 25 mL methanol for 30 min with vortex mixing every 15 min. Filter the mixture using filter paper and use the collected filtrate.

c. Chromatographic System

i. **Adsorbent:** TLC plate silica gel 60 F254 (20x10 cm)

ii. **Application volume:** 0.2 µL sprayed as 9 mm bands using suitable device

iii. **Relative humidity:** Condition the plate to a relative humidity of about 33% for 15 min using a suitable device.

iv. **Developing solvent system:** a mixture of chloroform, ethyl acetate, and formic acid (5:4:1)

v. **Developing distance:** 85 mm

vi. **Derivatization reagent:** 1% aluminum chloride in methanol

d. Analysis

i. **Samples:** *Standard Solution and Sample Solution* Apply the samples to the TLC plate and pre-dry. Develop the chromatogram in a chamber saturated for 20 min using a suitable device and remove the developed plate after drying. Examine under normal light, short wavelength UV light (254 nm), and long wavelength UV light (366 nm). Treat with derivatization reagent, and heat at 100 for 7 min. After cooling, examine again under normal light, short wavelength UV light (254 nm), and long wavelength UV light (366 nm).

ii. **Acceptance criteria:** The sequence of zones present in the chromatogram of the sample solution must exhibit similar pattern to the reference chromatogram. Other faint bands may be present in the chromatogram of the sample solution. See *Annex* for the reference chromatogram.

FOREIGN MATTER

<2> not more than 2%

LOSS ON DRYING

<3> not more than 10%

ALCOHOL (95% v/v)–SOLUBLE EXTRACTIVES

<4> not less than 3%

WATER-SOLUBLE EXTRACTIVES

<4> not less than 16%

TOTAL ASH

<5> not more than 18%

ACID-INSOLUBLE ASH

<5> not more than 8%

WATER-SOLUBLE ASH

<5> not more than 4%

TEST FOR TOXIC HEAVY METALS

<54> The toxic heavy metal limits should conform to the specified limits stated in AO 184 s. 2004. Sample should not have more than 0.3 ug/g (ppm) for arsenic and cadmium, 10 ug/g (ppm) for lead, and 0.5 ug/g (ppm) for mercury.

AFLATOXIN

<57> Total aflatoxin shall conform to the prescribed specification of 20 mcg/kg and aflatoxin B1 of 10 mcg/kg by ELISA method or liquid chromatography

PESTICIDE LIMITS

<56> shall conform to the limits defined by WHO/FAO Codex Alimentarius

MICROBIAL LIMIT

<52> The total aerobic microbial count does not exceed 10^5 cfu/g, the total combined molds and yeast count does not exceed 10^3 cfu/g, and it meets the requirements of the tests for the absence of *Salmonella* species and *Escherichia coli*. Other enterobacteria do not exceed 10^3 cfu/g.

TRADITIONAL USES

Traditionally used to treat stomachache, abdominal pain, diarrhea, preventing tooth decay, and disinfectant wash during childbirth. Also used as antinociceptive, antibacterial, analgesic, hepatoprotection, enhanced wound healing, prevent tooth decay, treatment of cough and skin disease, and antimutagenic.

Ehretiae Microphylla Tablet
Tsaang Gubat Tablet

DEFINITION

Ehretiae Microphylla Folium Pulveratum processed into tablet form. Each tablet of Ehretiae Microphylla may contain 250 mg or 500 mg of dried *tsaang gubat* leaf powder.

WARNING

There are no sufficient scientific studies done to investigate adverse reactions nor side effects of *tsaang gubat* tablets during pregnancy and breastfeeding.

PACKAGING AND STORAGE

Store at a temperature not exceeding 30˚C.

LABELING

Print the official title and the national title of the herbal material on the label as directed under *General Notices and Requirements* Part I.

UNIFORMITY OF DOSAGE UNITS

<31> meets the requirements.

DISINTEGRATION

<38> All of the tablets disintegrated completely with no palpable firm core in not more than 30 min.

FRIABILITY

<37> maximum mean weight loss from the samples is not more than 1%

MICROBIAL LIMIT

<52> The total aerobic microbial count does not exceed 10^5 cfu/g, the total combined molds and yeast count does not exceed 10^3 cfu/g, and it meets the requirements of the tests for the absence of *Salmonella* species and *Escherichia coli*. Other enterobacteria do not exceed 10^3 cfu/g.

MEDICAL USES

Treatment for gastrointestinal colic and biliary colic.

MENTHAE XVILLOSAE

Menthae XVillosae Folium
Yerba Buena Leaf

DEFINITION

Menthae XVillosae Folium consists of the dried leaf of *Mentha Xvillosa* Huds. (Fam. Lamiaceae).

SCIENTIFIC NAME

Mentha Xvillosa Huds.

SYNONYMS

Mentha Xbenthamiana Timb.-Lagr.
Mentha Xcordifolia Opiz ex Fresen
Mentha Xemarginata Rchb.
Mentha Xfloccida Déségl.
Mentha Xniliacea Vahl

SELECTED COMMON NAMES

yerba buena, *ablebana*, *herba buena*, *hierba buena*, *hilbas*, *polio* (Tag.); *daun poko* (Ind), *pohok* (Mal.); *apple mint*, *mojito mint*, *bowl mint*, *wooly mint* (Eng.)

MAJOR CHEMICAL CONSTITUENTS

Menthae [x]Villosae Folium collected from the Philippine hybrid yields a volatile oil containing piperitenone oxide as the principal constituent together with other 3-oxygenated monoterpenes. From the leaves, three compounds have been isolated, identified, and the biological activity of each has been determined. These are 6,7-bis-(2,2-dimethoxyethene)-(2, 11-dimethoxy-2Z,4E,8E, 10Z-dodecatetraenedioic acid (antimutagenic), β-sitosterol (analgesic, antimutagenic, anthelmintic), and β-sitosteryl-β-D-glucoside (analgesic).

MORPHOLOGICAL DESCRIPTION OF THE PLANT

The strongly aromatic, highly branching herb grows from 0.6 to 40 cm long, with smooth or partially hairy, usually purplish stem. Flowers, terminal or axillary, with four stamens, grow in capitate whorls, and are borne by a triangular or lanceolated hairy, campanulate/tubular five-toothed calyx and hairy four-lobed corolla. Fruit is a small, dry one seeded smooth or reticulate nutlet found at the base of the calyx.

MACROSCOPIC DESCRIPTION

Short petiolated leaves are attached to the stem. These are elliptic to oblong ovate in shape. The leaf margins are serrated, rounded or obtuse. Size ranges from 1.4 to 4 cm long.

MICROSCOPIC DESCRIPTION

The upper epidermal layer of the leaf of yerba buena has knob-like shaped cells. The layer is lined with a thin cuticle. Below the upper epidermis is a layer of elongated closely packed palisade cells with chloroplasts and loosely packed spongy cells above the lower epidermis. The lower epidermal layer consists of small rectangular cells. The midrib has undeveloped collenchymatous cells below the upper epidermis and above the lower epidermis. The main collateral vascular bundle is in the center of the predominant parenchymatous cells of the midrib.

WARNING

May trigger irritation of the fingers for sensitive people, which is due to the volatile oil.

PACKAGING AND STORAGE

Preserve in well-closed, light-resistant containers.

LABELING

Print the official title and the national title of the herbal material on the label as directed under *General Notices and Requirements* Part I.

TRADITIONAL USES

It is used for its antinociceptive property, antiseptic, anti-cancer, diuretic, anti-spasm, anti-emetic activities. Also used for treating arthtisis, stomach ache, flatulence, indigestion, headaches, and toothaches.

Menthae [x]Villosae Folium Pulveratum

Yerba Buena Leaf Powder

DEFINITION

Menthae [x]Villosae Folium reduced to powder form.

SCIENTIFIC NAME

Mentha [x]villosa Huds.

SYNONYMS

Mentha [x]benthamiana Timb.-Lagr.
Mentha [x]cordifolia Opiz ex Fresen
Mentha [x]emarginata Rchb.
Mentha [x]floccida Déségl.
Mentha [x]niliacea Vahl

SELECTED COMMON NAMES

yerba buena, *ablebana*, *herba buena*, *hierba buena*, *hilbas*, *polio* (Tag.); *daun poko* (Ind), *pohok* (Mal.); *apple mint*, *mojito mint*, *bowl mint*, *wooly mint* (Eng.)

MAJOR CHEMICAL CONSTITUENTS

Menthae [x]Villosae Folium collected from the Philippine hybrid yields a volatile oil containing piperitenone oxide as the principal constituent together with other 3-oxygenated monoterpenes. From the leaves, three compounds have been isolated, identified, and the biological activity of each has been determined. These are 6,7-bis-(2,2-dimethoxyethene)-(2, 11-dimethoxy-2Z,4E,8E, 10Z-dodecatetraenedioic acid (antimutagenic),

β-sitosterol (analgesic, antimutagenic, anthelmintic), and β-sitosteryl-β-D-glucoside (analgesic).

WARNING

Extended contact with *yerba buena* can cause skin irritation.

PACKAGING AND STORAGE

Preserve in well-closed, light-resistant containers.

LABELING

Print the official title and the national title of the herbal material on the label as directed under *General Notices and Requirements* Part I.

IDENTIFICATION

a. Sample Solution

Sonicate 2.5 g of 5 g of *Mentha ˣvillosa* Leaf, finely powdered, in 25 mL methanol for 30 min with vortex mixing every 15 min. Filter the mixture using filter paper and use the collected filtrate.

b. Chromatographic System

i. **Adsorbent:** TLC plate silica gel 60 F254 (20x10 cm)

ii. **Application volume:** 0.2 µL sprayed as 9 mm bands using suitable device

iii. **Relative humidity:** Condition the plate to a relative humidity of about 33% for 15 min using a suitable device.

iv. **Developing solvent system:** a mixture of chloroform and ethyl acetate (95:5)

v. **Developing distance:** 85 mm

vi. **Derivatization reagent:** Anisaldehyde-sulfuric acid reagent – Mix in order of 85 mL cold methanol, 10 mL glacial acetic acid, 0.5 mL p-anisaldehyde, and 5 mL concentrated sulfuric acid (perform in ice bath).

c. Analysis

i. **Samples:** *Sample Solution* Apply the samples to the TLC plate and pre-dry. Develop the chromatogram in a chamber saturated for 20 min using a suitable device and remove the developed plate after drying. Examine under normal light, short wavelength UV light (254 nm), and long wavelength UV light (366 nm). Treat with derivatization reagent, and heat at 100 for 7 min. After cooling, examine again under normal light, short wavelength UV light (254 nm), and long wavelength UV light (366 nm).

ii. **Acceptance criteria:** The sequence of zones present in the chromatogram of the sample solution must exhibit similar pattern to the reference chromatogram. Other faint bands may be present in the chromatogram of the sample solution. See *Annex* for the reference chromatogram.

FOREIGN MATTER

<2> not more than 2%

LOSS ON DRYING/MOISTURE CONTENT

<3> not more than 10%

ALCOHOL (95% v/v)–SOLUBLE EXTRACTIVES

<4> not less than 4%

WATER-SOLUBLE EXTRACTIVES

<4> not less than 15%

TOTAL ASH

<5> not more than 16%

ACID-INSOLUBLE ASH

<5> not more than 4%

WATER-SOLUBLE ASH

<5> not more than 8%

TEST FOR TOXIC HEAVY METALS

<54> The toxic heavy metal limits should conform to the specified limits stated in AO 184 s. 2004. Sample should not have more than 0.3 ug/g (ppm) for arsenic and cadmium, 10 ug/g (ppm) for lead, and 0.5 ug/g (ppm) for mercury.

AFLATOXIN

<57> Total aflatoxin shall conform to the prescribed specification of 20 mcg/kg and aflatoxin B1 of 10 mcg/kg by ELISA method or liquid chromatography

PESTICIDE LIMITS

<56> shall conform to the limits defined by WHO/FAO Codex Alimentarius

MICROBIAL LIMIT

<52> The total aerobic microbial count does not exceed 10^5 cfu/g, the total combined molds and yeast count does not exceed 10^3 cfu/g, and it meets the requirements of the tests for the absence of *Salmonella* species and *Escherichia coli.* Other enterobacteria do not exceed 10^3 cfu/g.

TRADITIONAL USES

It is used for its antinociceptive property, antiseptic, anti-cancer, diuretic, anti-spasm, anti-emetic activities. Also used for treating arthtisis, stomach ache, flatulence, indigestion, headaches, and toothaches.

Menthae ^{x}Villosae Tablet
Yerba Buena Tablet

DEFINITION

Menthae ^{x}Villosae Folium Pulveratum processed to tablet form. Each tablet of Menthae ^{x}Villosae contains 500 mg of dried *yerba buena* leaf powder.

WARNING

There are no sufficient studies made relating to side effects of *yerba buena* in pregnancy and breastfeeding.

PACKAGING AND STORAGE

Store at a temperature not exceeding 30˚C. Protect from moisture.

LABELING

Print the official title and the national title of the herbal material on the label as directed under *General Notices and Requirements* Part I.

UNIFORMITY OF DOSAGE UNITS

<31> meets the requirements.

DISINTEGRATION

<38> All of the tablets disintegrated completely with no palpable firm core in not more than 30 min.

FRIABILITY

<37> maximum mean weight loss from the samples is not more than 1%

MICROBIAL LIMIT

<52> The total aerobic microbial count does not exceed 10^5 cfu/g, the total combined molds and yeast count does not exceed 10^3 cfu/g, and it meets the requirements of the tests for the absence of *Salmonella* species and *Escherichia coli.* Other enterobacteria do not exceed 10^3 cfu/g.

MEDICAL USES

Used for the relief of pain post-excision biopsy, post-dental extraction, post-episiotomy, and post-circumcision.

MOMORDICAE CHARANTIAE

Momordicae Charantiae Folium
Ampalaya Leaf

DEFINITION

Momordicae Charantiae Folium is the dried leaf and leafy top of *Momordica charantia* L. 'Makiling' (Fam. Cucurbitaceae).

SCIENTIFIC NAME

Momordica charantia L.

SYNONYMS

Cucumis argyi H. Lev.
Cucumis intermedius M. Roem.
Momordica indica L.
Momordica muricata Willd.
Momordica sinensis Spreng.

SELECTED COMMON NAMES

Bitter gourd, *bitter cucumber, balsam pear* (En). *Bitter melon* (Am). *Margose*, *paroka* (Fr). Indonesia: *paria* (general), *pare* (Javanese), *papari* (Moluccas). Malaysia: *peria, peria laut, periok.* Philippines: *ampalaya* (Tagalog), *paria* (Ilocano), *palia* (Bisaya). Cambodia: *mreah*. Laos: *'hai1, 'phak 'ha, sai1*. Thailand: *mara* (general), *maha* (northern), *phakha* (north-eastern). Vietnam: *m[uw] [ows] p d[aws]ng, m[uw] [ows]p m[ur]* (northern), *kh[oor] qua* (south).

MAJOR CHEMICAL CONSTITUENTS

Momordicae Charantiae Folium contains charantin (momorcharin) which is a 1:1 mixture of stigmastadienol β-D-glucoside and β-sitosterol β-D-glucoside. It has triterpenoids belonging mostly to cucurbitane group and are mainly glucosides such as momordicisides and momorcharasides. The folium also contains cucurbitacins called kuguacins F-S.

MORPHOLOGICAL DESCRIPTION OF THE PLANT

Momordica charantia is a climbing herbaceous vine, somewhat not hairy. Leaves are cut into five to seven lobes near the base. These have simple tendrils, stretching up to 20 cm long, that cling or wind around an object, to support the entire plant. Yellow flowers are axillary and are borne by long peduncles. It has the pistil and stamen borne in separate flowers (dioecious). The male flower, supported by a peduncle reaching approximately 12 mm long, has a rounded green bract approximately 1 cm towards the middle. The female flowers have longer peduncles. The fruit is oblong shaped, cylindric, ribbed and wrinkled, measuring 15-25 cm long and is pointed at both ends. Similarly, the seeds are also oblong, compressed, corrugated on the margins and are approximately 10-15 mm long.

MACROSCOPIC DESCRIPTION

The leaves are rounded or orbicular, measuring 2.5 - 10 cm in diameter, that cut nearly to the base, essentially forming five or seven oblong ovate or variously toothed lobes. These have leaf bases that are heart-shaped (cordate).

MICROSCOPIC DESCRIPTION

The leaf of *Momordica charantia* has a uniseriate layer of cells in both upper and lower epidermis. Sometimes epidermal hairs are present as glandular, long multicellular cells. The mesophyll layer beneath the epidermis is composed of elongated palisade cells (found adjacent to the upper epidermis) and irregularly shaped spongy cells interspersed with tiny veinlets (near the lower epidermis). The midrib area has collenchymatous cells beneath the upper epidermis. The former has large parenchymatous cells with lignified xylem and parenchymatous phloem cells, forming the upper and lower part of the vascular bundle respectively.

WARNING

May trigger allergic reactions for sensitive skin.

PACKAGING AND STORAGE

Preserve in well-closed, light-resistant containers.

LABELING

Print the official title and the national title of the herbal material on the label as directed under *General Notices and Requirements* Part I.

TRADITIONAL USES

Treatment for cough, colds, asthma, emetic for the newborn, colic, stomach ache, diabetes, anemia, fever, malaria, ringworm, and wounds. Also used for antidiabetic, antioxidant, anti-inflammatory, laxative, hepatoprotective, immunobooster, anti-gout, anti-rheumatic, antipyretic, astringent, analgesic, anthelmintic, antimicrobial, anti-cancer, anti-psoriatic, anti-obesity, and appetite stimulant.

Momordicae Charantiae Folium Pulveratum
Ampalaya Leaf Powder

DEFINITION

Momordicae Charantiae Folium reduced to powder form.

SCIENTIFIC NAME

Momordica charantia L.

SYNONYMS

Cucumis argyi H. Lev.
Cucumis intermedius M. Roem.
Momordica indica L.
Momordica muricata Willd.
Momordica sinensis Spreng.

SELECTED COMMON NAMES

Bitter gourd, *bitter cucumber, balsam pear* (En). *Bitter melon* (Am). *Margose*, *paroka* (Fr). Indonesia: *paria* (general), *pare* (Javanese), *papari* (Moluccas). Malaysia: *peria, peria laut, periok.* Philippines: *ampalaya* (Tagalog), *paria* (Ilocano), *palia* (Bisaya). Cambodia: *mreah*. Laos: *'hai1, 'phak 'ha, sai1*. Thailand: *mara* (general), *maha* (northern), *phakha* (north-eastern). Vietnam: *m[uw] [ows] p d[aws]ng, m[uw] [ows]p m[ur]* (northern), *kh[oor] qua* (south).

MAJOR CHEMICAL CONSTITUENTS

Momordicae Charantiae Folium Pulveratum contains charantin (momorcharin) which is a 1:1 mixture of stigmastadienol β-D-glucoside and β-sitosterol β-D-glucoside. It has triterpenoids belonging mostly to cucurbitane group and are mainly glucosides such as momordicisides and momorcharasides. The folium also contains cucurbitacins called kuguacins F-S.

WARNING

May trigger allergic reactions for sensitive skin.

PACKAGING AND STORAGE

Preserve in well-closed, light-resistant containers.

LABELING

Print the official title and the national title of the herbal material on the label as directed under *General Notices and Requirements* Part I.

IDENTIFICATION

a. Standard Solution

0.25 mg/L of β-Sitosterol glucoside RS in methanol. Sonicate for 1 min and mix by vortex.

b. Sample Solution

Sonicate 2.5 g of *Momordica charantia* Leaf, finely powdered, in 25 mL methanol for 30 min with vortex mixing every 15 min. Filter the mixture using filter paper and use the collected filtrate.

c. Chromatographic System

i. **Adsorbent:** TLC plate silica gel 60 F254 (20x10 cm)

ii. **Application volume:** 0.2 µL sprayed as 9 mm bands using suitable device

iii. **Relative humidity:** Condition the plate to a relative humidity of about 33% for 15 min using a suitable device.

iv. **Developing solvent system:** a mixture of chloroform, ethyl acetate, and formic acid (5:7.5:0.75)

v. **Developing distance:** 85 mm

vi. **Derivatization reagent:** Anisaldehyde-sulfuric acid reagent – Mix in order of 85 mL cold methanol, 10 mL glacial acetic acid, 0.5 mL p-anisaldehyde, and 5 mL concentrated sulfuric acid (perform in ice bath).

d. Analysis

i. **Samples:** *Standard Solution and Sample Solution* Apply the samples to the TLC plate and pre-dry. Develop the chromatogram in a chamber saturated for 20 min using a suitable device and remove the developed plate after drying. Examine under normal light, short wavelength UV light (254 nm), and long wavelength UV light (366 nm). Treat with derivatization reagent, and heat at 100 for 7 min. After cooling, examine again under normal light, short wavelength UV light (254 nm), and long wavelength UV light (366 nm).

ii. **Acceptance criteria:** The sequence of zones present in the chromatogram of the sample solution must exhibit similar pattern to the reference chromatogram. Other faint bands may be present in the chromatogram of the sample solution. See *Annex* for the reference chromatogram.

FOREIGN MATTER

<2> not more than 2%

LOSS ON DRYING/MOISTURE CONTENT

<3> not more than 10%

ALCOHOL (95% v/v)–SOLUBLE EXTRACTIVES

<4> not less than 5%

WATER-SOLUBLE EXTRACTIVES

<4> not less than 22%

TOTAL ASH

<5> not more than 14%

ACID-INSOLUBLE ASH

<5> not more than 5%

WATER-SOLUBLE ASH

<5> not more than 7%

TEST FOR TOXIC HEAVY METALS

<54> The toxic heavy metal limits should conform to the specified limits stated in AO 184 s. 2004. Sample should not have more than 0.3 ug/g (ppm) for arsenic and cadmium, 10 ug/g (ppm) for lead, and 0.5 ug/g (ppm) for mercury.

AFLATOXIN

<57> Total aflatoxin shall conform to the prescribed specification of 20 mcg/kg and aflatoxin B1 of 10 mcg/kg by ELISA method or liquid chromatography

PESTICIDE LIMITS

<56> shall conform to the limits defined by WHO/FAO Codex Alimentarius

MICROBIAL LIMIT

<52> The total aerobic microbial count does not exceed 10^5 cfu/g, the total combined molds and yeast count does not exceed 10^3 cfu/g, and it meets the requirements of the tests for the absence of *Salmonella* species and *Escherichia coli.* Other enterobacteria do not exceed 10^3 cfu/g.

TRADITIONAL USES

Treatment for cough, colds, asthma, emetic for the newborn, colic, stomach ache, diabetes, anemia, fever, malaria, ringworm, and wounds. Also used for antidiabetic, antioxidant, anti-inflammatory, laxative, hepatoprotective, immunobooster, anti-gout, anti-rheumatic, antipyretic, astringent, analgesic, anthelmintic, antimicrobial, anti-cancer, anti-psoriatic, anti-obesity, and appetite stimulant.

Momordicae Charantiae Tablet
Ampalaya Tablet

DEFINITION

Momordicaea Charantiae Folium Pulveratum processed to tablet form. Each tablet of Momordicaea Charantiae contains 500 mg of dried *ampalaya* leaf powder.

WARNING

Concomitant hypoglycemic medications. Do not use *ampalaya* tablet medications in pregnancy. Taking *ampalaya* in medicinal doses may counteract or aggravate its effectiveness.

PACKAGING AND STORAGE

Store at temperature not exceeding 30˚C. Protect from moisture.

LABELING

Print the official title and the national title of the herbal material on the label as directed under *General Notices and Requirements* Part I.

UNIFORMITY OF DOSAGE UNITS

<31> meets the requirements.

DISINTEGRATION

<38> All of the tablets disintegrated completely with no palpable firm core in not more than 30 min.

FRIABILITY

<37> maximum mean weight loss from the samples is not more than 1%

MICROBIAL LIMIT

<52> The total aerobic microbial count does not exceed 10^5 cfu/g, the total combined molds and yeast count does not exceed 10^3 cfu/g, and it meets the requirements of the tests for the absence of *Salmonella* species and *Escherichia coli.* Other enterobacteria do not exceed 10^3 cfu/g.

MEDICAL USES

Antidiabetic agent for Type 2 diabetes mellitus.

PLECTRANTHI AMBOINICI

Plectranthi Amboinici Folium
Oregano Leaf

DEFINITION

Plectranthi Amboinici Folium is the dried leaf of *Plectranthus amboinicus* (Lour.) Spreng. (Fam. Labiatae/ Lamiaceae).

SCIENTIFIC NAME

Plectranthus amboinicus (Lour.) Spreng.

SYNONYMS

Coleus amboinicus Lour.
Coleus aromaticus Benth.
Coleus carnosus Hassk.
Coleus crassifolius Benth
Coleus subfrutectosus Summerh.

SELECTED COMMON NAMES

Filipino: *badlaja* (Buk.), *bildo* (Sul., Taw.), *bildu* (Sul.), *carabo* (Sam., Ley.), *clavo* (Ak., Cam., Dav., Mas., Mis., Sor.), *clavo de comer* (Cap.), *latai* (Sul.), *oregano*, *suganda* (Tag.), *torongil de limon* (Spa.), Country *borage*, *Indian borage* (En). Indonesia: *daun jinten* (general), *ajeran* (Sundanese), *daun kucing* (Javanese). Malaysia: *bangun-bangun*, *membangun* (Peninsular). (Sp), *suganda* (Tagalog), *latai* (Subanun). Cambodia: *sak dam ray*. Thailand: *niam hu suea* (central), *hom duan luang, hom duan hu suea* (northern). Vietnam: *rau t[aaf]n*, *rau t[aaf]n d[aaf]y l[as]*, *h[us]ng chanh*

MAJOR CHEMICAL CONSTITUENTS

Plectranthi Amboinici Folium contains monoterpene hydrocarbons and oxygenated monoterpenes, sesquiterpenes, and oxygenated sesquiterpenes including carvacrol, β-caryophyllene, -cymene, -terpinene, -bergamotene, -caryophyllene, eudesma-4,11-diene, 4-terpineol, -cubebene, and caryophyllene oxide.

MORPHOLOGICAL DESCRIPTION OF THE PLANT

Plectranthus amboinicus is an erect or spreading branched strongly aromatic green herbaceous plant where the stem is fleshy. Flowers small in distant whorls. The bell-shaped calyx has a smooth two lipped throat inside, with a thin ovate upper lip and four narrow toothed lower lips. The pale purplish corolla is five times longer than the calyx, appearing as a short tube, inflated throat and short lips.

MACROSCOPIC DESCRIPTION

Leaves are fleshy, with hairs on the veins and petiole, broadly ovate, often heart-shaped, acute apex, obtuse base, margins crenate with soft minute hairs. Leaves prolonged on the stem beneath their insertion. Prominent veins at the lower surface, pinnate venation.

MICROSCOPIC DESCRIPTION

The upper epidermis of the leaf of oregano has a thin and smooth cuticle covering its layer of small thin-walled rectangular cells where in surface view are polygonal with straight or slightly sinuous vertical walls. It has numerous diacytic stomata (two subsidiary cells surround the guard cell). Near the midrib, the mesophyll layer has several layers of near rounded closely packed parenchymatous cells with chloroplastids and oil globules. The major bulk of the mesophyll layer (the lamina portion) is made up of a few layers of closely packed short columnar palisade cells and rounded spongy parenchyma. Calcium oxalate crystals, specifically rosette and prismatic types, are found mostly in the parenchyma cells of the midrib. The lower epidermis is composed of a single layer of rectangular thin walled cells with stomata. Near the midrib, the mesophyll tissues has several layers of nearly rounded closely packed parenchymatous cells with chloroplastids and oil globules. In the midrib, few collenchyma cells can be found beneath the upper epidermis and above the lower epidermis. Collateral vascular bundle (where phloem and xylem strands are in the same radius) lie at the center of the predominantly parenchymatous cells of the midrib. Two glandular trichomes are present: one with a short one-celled stalk and on to two-celled sub-globular glandular head and the second has a longer two-celled stalk and one-celled glandular head with oil contents. Two-celled non-glandular trichomes are also present.

WARNING

May trigger irritation for sensitive people, which is due to the oregano oil.

PACKAGING AND STORAGE

Preserve in well-closed, light-resistant containers.

LABELING

Print the official title and the national title of the herbal material on the label as directed under *General Notices and Requirements* Part I.

TRADITIONAL USES

Traditionally used as an anti-oxidant, antimicrobial, anti-inflammatory, and analgesic. Oregano extract and its essential oil can be used as a natural food preservative. Traditionally used on treating asthma, cramps, diarrhea, and indigestion. Also used for colds and stomach upset and has antimicrobial property.

Plectranthi Amboinici Folium Pulveratum
Oregano Leaf Powder

DEFINITION

Plectranthi Amboinici Folium Pulveratum reduced to powder form.

SCIENTIFIC NAME

Plectranthus amboinicus (Lour.) Spreng.

SYNONYMS

Coleus amboinicus Lour.
Coleus aromaticus Benth.
Coleus carnosus Hassk.
Coleus crassifolius Benth
Coleus subfrutectosus Summerh.

SELECTED COMMON NAMES

Filipino: *badlaja* (Buk.), *bildo* (Sul., Taw.), *bildu* (Sul.), *carabo* (Sam., Ley.), *clavo* (Ak., Cam., Dav., Mas., Mis., Sor.), *clavo de comer* (Cap.), *latai* (Sul.), *oregano*, *suganda* (Tag.), *torongil de limon* (Spa.), Country *borage*, *Indian borage* (En). Indonesia: *daun jinten* (general), *ajeran* (Sundanese), *daun kucing* (Javanese). Malaysia: *bangun-bangun*, *membangun* (Peninsular). (Sp), *suganda* (Tagalog), *latai* (Subanun). Cambodia: *sak dam ray*. Thailand: *niam hu suea* (central), *hom duan luang, hom duan hu suea* (northern). Vietnam: *rau t[aaf]n*, *rau t[aaf]n d[aaf]y l[as]*, *h[us]ng chanh*

MAJOR CHEMICAL CONSTITUENTS

Plectranthi Amboinici Folium Pulveratum contains monoterpene hydrocarbons and oxygenated monoterpenes, sesquiterpenes, and oxygenated sesquiterpenes including carvacrol, β-caryophyllene, -cymene, -terpinene, -bergamotene, -caryophyllene, eudesma-4,11-diene, 4-terpineol, -cubebene, and caryophyllene oxide.

WARNING

May trigger irritation for sensitive people due to the oregano oil.

PACKAGING AND STORAGE

Preserve in well-closed, light-resistant containers. Protect from moisture.

LABELING

Print the official title and the national title of the herbal material on the label as directed under *General Notices and Requirements* Part I.

IDENTIFICATION

a. Standard Solution

2.0 mg/mL of Carvacrol RS in methanol. Sonicate for 1 min and mix by vortex.

b. Sample Solution

Sonicate 2.5 g *Plectranthus amboinicus* Leaf, finely powdered, in 25 mL methanol for 30 min with vortex mixing every 15 min. Filter the mixture using filter paper and use the collected filtrate.

c. Chromatographic System

i. **Adsorbent:** TLC plate silica gel 60 F254 (20x10 cm)

ii. **Application volume:** 0.2 µL sprayed as 9 mm bands using suitable device

iii. **Relative humidity:** Condition the plate to a relative humidity of about 33% for 15 min using a suitable device.

iv. **Developing solvent system:** a mixture of hexane, ethyl acetate and glacial acetic acid (1:1:0.05)

v. **Developing distance:** 85 mm

vi. **Derivatization reagent:** Anisaldehyde-sulfuric acid reagent – Mix in order of 85 mL cold methanol, 10 mL glacial acetic acid, 0.5 mL p-anisaldehyde, and 5 mL concentrated sulfuric acid (perform in ice bath).

d. Analysis

i. **Samples:** *Standard Solution and Sample Solution* Apply the samples to the TLC plate and pre-dry. Develop the chromatogram in a chamber saturated for 20 min using a suitable device and remove the developed plate after drying. Examine under normal light, short wavelength UV light (254 nm), and long wavelength UV light (366 nm).

Treat with derivatization reagent, and heat at 100 for 7 min. After cooling, examine again under normal light, short wavelength UV light (254 nm), and long wavelength UV light (366 nm).

ii. **Acceptance criteria:** The sequence of zones present in the chromatogram of the sample solution must exhibit similar pattern to the reference chromatogram. Other faint bands may be present in the chromatogram of the sample solution. See *Annex* for the reference chromatogram.

FOREIGN MATTER

<2> not more than 2%

LOSS ON DRYING/MOISTURE CONTENT

<3> not more than 10%

ALCOHOL (95% v/v)–SOLUBLE EXTRACTIVES

<4> not less than 5%

WATER-SOLUBLE EXTRACTIVES

<4> not less than 22%

TOTAL ASH

<5> not more than 28%

ACID-INSOLUBLE ASH

<5> not more than 5%

WATER-SOLUBLE ASH

<5> not more than 7%

TEST FOR TOXIC HEAVY METALS

<54> The toxic heavy metal limits should conform to the specified limits stated in AO 184 s. 2004. Sample should not have more than 0.3 ug/g (ppm) for arsenic and cadmium, 10 ug/g (ppm) for lead, and 0.5 ug/g (ppm) for mercury.

AFLATOXIN

<57> Total aflatoxin shall conform to the prescribed specification of 20 mcg/kg and aflatoxin B1 of 10 mcg/kg by ELISA method or liquid chromatography

PESTICIDE LIMITS

<56> shall conform to the limits defined by WHO/FAO Codex Alimentarius

MICROBIAL LIMIT

<52> The total aerobic microbial count does not exceed 10^5 cfu/g, the total combined molds and yeast count does not exceed 10^3 cfu/g, and it meets the requirements of the tests for the absence of *Salmonella* species and *Escherichia coli.* Other enterobacteria do not exceed 10^3 cfu/g.

TRADITIONAL USES

Traditionally used as an anti-oxidant, antimicrobial, anti-inflammatory, and analgesic. *Oregano* extract and its essential oil can be used as a natural food preservative. Traditionally used on treating asthma, cramps, diarrhea, and indigestion. Also used for colds and stomach upset and has antimicrobial property.

PEPEROMIAE PELLUCIDAE

Peperomiae Pellucidae Herba
Ulasimang Bato Aerial Parts

DEFINITION

Peperomiae Pellucidae Herba is the dried aerial parts of *Peperomia pellucida* (L.) Kunth.

SCIENTIFIC NAME

Peperomia pellucida (L.) Kunth.

SYNONYMS

Micropiper pellucidum (L.) Miq.
Peperomia concinna (Haw.) A. Dietr.
Peperomia knoblecheriana Schott
Piper pellucidum L.

SELECTED COMMON NAMES

Indonesia: *ketumpangan air* (Malay, Sumatra), *sasaladaan* (Sundanese), *suruh-suruhan* (Javanese). Malaysia: *ketumpangan air* (Malay, Peninsular). Philippines: *ulasiman-bato* (Tagalog), *olasiman-ihalas* (Cebu Bisaya), *tangon-tangon* (Bikol). Thailand: *chaa kruut* (peninsular), *phak krasang* (central), *phak haak kluai* (northern). Vietnam: *rau c[af]ng cua*.

MAJOR CHEMICAL CONSTITUENTS

Peperomiae Pellucidae Herba contains essential oils mostly composed of dillapiole, (E)-caryophyllene, and carotol.

MORPHOLOGICAL DESCRIPTION OF THE PLANT

The very succulent erect pale green herb has a height of 5-40 cm. Inflorescence (flower clusters) are terminal spikes that are also green, erect, slender, measuring 1-6 cm long. Florets are small, bracteate, perfect flowers with two stamens and with one-celled ovule within a one-celled ovary. Fruit is a small, brownish, globose drupe.

MACROSCOPIC DESCRIPTION

The pale green-colored stems are rounded, usually 5 mm thick. The leaves are also pale green in color and are transparent (pellucid) and shining. The leaves are simple, alternate, entire, with ovate, obtuse, acute heart shape where leaf base is rounded and heart-shaped. Leaf size ranges from 1-3 cm long.

MICROSCOPIC DESCRIPTION

Cuboidal epidermal cells line the outermost layer in the stem transverse section. Small cuboidal cells as well are found between the epidermis and the ground tissue. Druse and rosette crystals are numerously scattered in the epidermal and parenchymatous ground tissue. The vascular tissues are located within the ground tissue.

Cutinized epidermal layer with irregularly shaped cells lines the outermost layer of the leaf blade cross section. Large parenchymatous mesophyll tissues are filled up with many chloroplasts and prismatic crystals. Helical vessels are the main water conducting tissues located in the mesophyll area. Stomata are only found in the lower epidermis (hypostomatic).

WARNING

May trigger allergic reactions for sensitive skin.

PACKAGING AND STORAGE

Preserve in well-closed, light-resistant containers.

LABELING

Print the official title and the national title of the herbal material on the label as directed under *General Notices and Requirements* Part I.

TRADITIONAL USES

Traditionally used for treating gout, arthritis, and UTI. Also used for skin allergies, psoriasis, itchiness, scabies, eczema, stomach ache, abdominal pain, and diarrhea.

Peperomiae Pellucidae Herba Pulveratum

Ulasimang Bato Aerial Part Powder

DEFINITION

Pereromiae Pellucidae Herba reduced to powder form.

SCIENTIFIC NAME

Peperomia pellucida (L.) Kunth.

SYNONYMS

Micropiper pellucidum (L.) Miq.
Peperomia concinna (Haw.) A. Dietr.
Peperomia knoblecheriana Schott
Piper pellucidum L.

SELECTED COMMON NAMES

Indonesia: *ketumpangan air* (Malay, Sumatra), *sasaladaan* (Sundanese), *suruh-suruhan* (Javanese). Malaysia: *ketumpangan air* (Malay, Peninsular). Philippines: *ulasiman-bato* (Tagalog), *olasiman-ihalas* (Cebu Bisaya), *tangon-tangon* (Bikol). Thailand: *chaa kruut* (peninsular), *phak krasang* (central), *phak haak kluai* (northern). Vietnam: *rau c[af]ng cua*.

MAJOR CHEMICAL CONSTITUENTS

Peperomiae Pellucidae Herba Pulveratum contains essential oils mostly composed of dillapiole, (E)-caryophyllene, and carotol.

WARNING

May trigger allergic reactions for sensitive skin.

PACKAGING AND STORAGE

Preserve in well-closed, light-resistant containers.

LABELING

Print the official title and the national title of the herbal material on the label as directed under *General Notices and Requirements* Part I.

IDENTIFICATION

a. Standard Solution

1.5 mg/mL of Dillapiole RS in ethanol. Sonicate for 1 min and mix by vortex.

b. Sample Solution

Sonicate 5 g of *Peperomia pellucida* aerial parts, finely powdered, in 25 mL methanol for 30 min with vortex mixing every 15 min. Filter the mixture using filter paper and use the collected filtrate.

c. Chromatographic System

i. **Adsorbent:** TLC plate silica gel 60 F254 (20x10 cm)

ii. **Application volume:** 0.2 µL sprayed as 9 mm bands using suitable device

iii. **Relative humidity:** Condition the plate to a relative humidity of about 33% for 15 min using a suitable device.

iv. **Developing solvent system:** a mixture of hexane, ethyl acetate, chloroform, and glacial acetic acid (3:1:1:0.025)

v. **Developing distance:** 85 mm

vi. **Derivatization reagent:** Anisaldehyde-sulfuric acid reagent – Mix in order of 85 mL cold methanol, 10 mL glacial acetic acid, 0.5 mL p-anisaldehyde and 5 mL concentrated sulfuric acid (perform in ice bath).

d. Analysis

i. **Samples:** *Standard Solution and Sample Solution*
Apply the samples to the TLC plate and pre-dry. Develop the chromatogram in a chamber saturated for 20 min using a suitable device and remove the developed plate after drying. Examine under normal light, short wavelength UV light (254 nm), and long wavelength UV light (366 nm). Treat with derivatization reagent, and heat at 100 for 7 min. After cooling, examine again under normal light, short wavelength UV light (254 nm), and long wavelength UV light (366 nm).

ii. **Acceptance criteria:** The sequence of zones present in the chromatogram of the sample solution must exhibit similar pattern to the reference chromatogram. Other faint bands may be present in the chromatogram of the sample solution. See *Annex* for the reference chromatogram.

FOREIGN MATTER

<2> not more than 2%

LOSS ON DRYING

<3> not more than 10%

ALCOHOL (95% v/v)–SOLUBLE EXTRACTIVES

<4> not less than 5%

WATER-SOLUBLE EXTRACTIVES

<4> not less than 25%

TOTAL ASH

<5> not more than 27%

ACID-INSOLUBLE ASH

<5> not more than 8%

WATER-SOLUBLE ASH

<5> not more than 16%

TEST FOR TOXIC HEAVY METALS

<54> The toxic heavy metal limits should conform to the specified limits stated in AO 184 s. 2004. Sample should not have more than 0.3 ug/g (ppm) for arsenic and cadmium, 10 ug/g (ppm) for lead, and 0.5 ug/g (ppm) for mercury.

AFLATOXIN

<57> Total aflatoxin shall conform to the prescribed specification of 20 mcg/kg and aflatoxin B1 of 10 mcg/kg by ELISA method or liquid chromatography

PESTICIDE LIMITS

<56> shall conform to the limits defined by WHO/FAO Codex Alimentarius

MICROBIAL LIMIT

<52> The total aerobic microbial count does not exceed 10^5 cfu/g, the total combined molds and yeast count does not exceed 10^3 cfu/g, and it meets the requirements of the tests for the absence of *Salmonella* species and *Escherichia coli.* Other enterobacteria do not exceed 10^3 cfu/g.

TRADITIONAL USES

Traditionally used for treating gout, arthritis, and UTI. Also used for skin allergies, psoriasis, itchiness, scabies, eczema, stomach ache, abdominal pain, and diarrhea.

Peperomiae Pellucidae Tablet
Ulasimang Bato Tablet

DEFINITION

Peperomiae Pellucidae Herba Pulveratum processed to tablet form. Each tablet of Peperomiae Pellucidae contains 500mg of dried *ulasimang bato* aerial parts powder.

WARNING

More research is needed on *ulasimang bato* to ascertain its safety when taken by pregnant women or breastfeeding mothers.

PACKAGING AND STORAGE

Store at a temperature not exceeding 30˚C. Protect from heat, light and moisture.

LABELING

Print the official title and the national title of the herbal material on the label as directed under *General Notices and Requirements* Part I.

UNIFORMITY OF DOSAGE UNITS

<31> meets the requirements for weight variation test

DISINTEGRATION

<38> All of the tablets disintegrated completely with no

palpable firm core in not more than 30 min.

FRIABILITY

<37> maximum mean weight loss from the samples is not more than 1%

MICROBIAL LIMIT

<52> The total aerobic microbial count does not exceed 10^5 cfu/g, the total combined molds and yeast count does not exceed 10^3 cfu/g, and it meets the requirements of the tests for the absence of *Salmonella* species and *Escherichia coli.* Other enterobacteria do not exceed 10^3 cfu/g.

MEDICAL USE

Antiurolithiasis agent

SENNAE ALATAE

Sennae Alatae Folium
Akapulko Leaf

DEFINITION

Sennae Alatae Folium is the dried leaflet of *Senna alata* (L.) Roxb. (Fam. Leguminosae/Fabaceae).

SCIENTIFIC NAME

Senna alata (L.) Roxb.

SYNONYMS

Cassia alata L.
Cassia bracteata L.f.
Cassia herpetica Jacq.
Herpetica rumphiana (DC.) J.Presl.
Herpetica alata (L.) Raf.

SELECTED COMMON NAMES

Filipino: *adadasi* (Ting.), *ancharasi* (Ig.), *andadasi*. *andadasi-dadakkel*, a*ndadasi-ng-bugbugtog* (Ilk.), *andalam* (Sul., Taw., Zambo.), *andalan* (Sul.), *bayabasin* (Bik., Bis., Tag.), *Bayabasbayabasan* (Tag.), *kapurko* (Tag.), *kasitas* (Bik., Bis.), *katanda* (Tag.), *katandang aso* (Tag.), *lengua* (Bat.), *pakagonkon* (Tag.), *pakayomkon kastila* (Pamp.), *palad* (Cap.), *palochina* (Bis.), *plutchina* (Cap., Gul., Ilo.), *yi bing jue ming* (Chi.), *emperor's candlesticks, ringworm bush or shub* (Eng.); *kasshia arata*, *kyandorubusshu* (Jap.), *gelenggang besar* (Mal.); *bajagua*, *guajavo*, *hierba de playa* (Spa.), *chumhet thet* (Thai.)

MAJOR CHEMICAL CONSTITUENTS

Sennae Alatae Folium contains the active glycosides, sennosides A, B, C, and D. Also present are rhein, emodin, aloeemodin, chrysophanol, physcion, free or as their glycosides. Kaempferol, a flavonoid, and β-sitosterol have also been identified.

MORPHOLOGICAL DESCRIPTION OF THE PLANT

Senna alata is an erect or coarse branched shrub, reaching a height of 1.5 to 5m, with prominently alternate, simple, pinnately compound leaves where leaves are without glands on the rachis. Inflorescence is a simple or panicled raceme, 10 to 50 cm long, where cluster of brightly yellow colored florets are borne terminally and axillary. Floral bracts are yellow, supporting the bright yellow flowers/ florets, about 4 cm in diameter. The fruit is a winged pod, approximately 15 cm long, which is tetragonal in shape and thick, smooth without hairs, and winged, septated, colored dark brown or nearly black, containing 50 to 60 flattened triangular seeds.

MACROSCOPIC DESCRIPTION

Leaves are 40-60 cm long with reticulate venation and leaflets ranging from 5 to 18 long, 3-7 cm wide. Leaf shape is oblong to obovate, elliptic, broad and rounded,

and minutely mucronate at the apex. Leaf base is oblique, margin entire, rigidly leathery, obscurely downy beneath.

MICROSCOPIC DESCRIPTION

Transverse section of the leaf shows epidermal cells lined with a cuticle layer, slightly sinuous walls on both sides. Papillae as epidermal appendages, blurred and rarely seen at the upper epidermis but clearly and densely seen at the lower epidermis. Occasionally, unicellular trichomes with thickened and slightly warty walls, are found on the lamina, but more frequently these are found at the lower surface of the midrib. Stomata have two subsidiary cells running parallel to the guard cells and are found in both lower and upper epidermis (i.e. paracytic and amphistomatic stomata). Beneath the upper epidermis are columnar cells making up the palisade layer and irregularly shaped spongy cells lie before the lower epidermis. Collenchyma cells are observed at the midrib. Sclerenchymatous bundle sheath surrounds the xylem and phloem of the vascular bundle.

WARNING

Akapulko have been reported to cause allergic reactions in sensitive people.

PACKAGING AND STORAGE

Preserve in well-closed, light-resistant containers.

LABELING

Print the official title and the national title of the herbal material on the label as directed under *General Notices and Requirements* Part I.

TRADITIONAL USES

It is used as an abortifacient, laxative, for parturition, anemia, constipation, dermatitis, dyspepsia, fever, diuretic, insecticide, treatment for skin diseases (e.g. ringworm, skin sores, sabies, etc.), snake bite, larvicide, and treatment for asthma and bronchitis.

Sennae Alatae Folium Pulveratum

Akapulko Leaf Powder

DEFINITION

Sennae Alatae Folium reduced to powder form.

SCIENTIFIC NAME

Senna alata (L.) Roxb.

SYNONYMS

Cassia alata L.
Cassia bracteata L.f.
Cassia herpetica Jacq.
Herpetica rumphiana (DC.) J.Presl.
Herpetica alata (L.) Raf.

SELECTED COMMON NAMES

Filipino: *adadasi* (Ting.), *ancharasi* (Ig.), *andadasi*. *andadasi-dadakkel*, a*ndadasi-ng-bugbugtog* (Ilk.), *andalam* (Sul., Taw., Zambo.), *andalan* (Sul.), *bayabasin* (Bik., Bis., Tag.), *Bayabasbayabasan* (Tag.), *kapurko* (Tag.), *kasitas* (Bik., Bis.), *katanda* (Tag.), *katandang aso* (Tag.), *lengua* (Bat.), *pakagonkon* (Tag.), *pakayomkon kastila* (Pamp.), *palad* (Cap.), *palochina* (Bis.), *plutchina* (Cap., Gul., Ilo.), *yi bing jue ming* (Chi.), *emperor's candlesticks, ringworm bush or shub* (Eng.); *kasshia arata*, *kyandorubusshu* (Jap.), *gelenggang besar* (Mal.); *bajagua*, *guajavo*, *hierba de playa* (Spa.), *chumhet thet* (Thai.)

MAJOR CHEMICAL CONSTITUENTS

Sennae Alatae Folium contains the active glycosides, sennosides A, B, C, and D. Also present are rhein, emodin, aloeemodin, chrysophanol, physcion, free or as their glycosides. Kaempferol, a flavonoid, and β-sitosterol have also been identified.

WARNING

Akapulko has been reported to cause allergic reactions in sensitive people.

PACKAGING AND STORAGE

Preserve in well-closed, light-resistant containers.

LABELING

Print the official title and the national title of the herbal material on the label as directed under *General Notices and Requirements* Part I.

IDENTIFICATION

a. Standard Solution

2.0 mg/mL of Senna Leaves RS in methanol. Sonicate for 1 min and mix by vortex.

b. Sample Solution

Sonicate 5 g of *Senna alata* Leaf, finely powdered, in 25 mL methanol for 30 min with vortex mixing every 15 min. Filter the mixture using filter paper and use the collected filtrate.

c. Chromatographic System

i. **Adsorbent:** TLC plate silica gel 60 F254 (20x10 cm)

ii. **Application volume:** 0.2 µL sprayed as 9 mm bands using suitable device

iii. **Relative humidity:** Condition the plate to a relative humidity of about 33% for 15 min using a suitable device.

iv. **Developing solvent system:** a mixture of propanol, ethyl acetate, water, and glacial acetic acid (30:50:30:3)

v. **Developing distance:** 85 mm

vi. **Derivatization reagent:** Spray 20% nitric acid and heat at 120°C for 15 min. Spray 50g/L potassium hydroxide in 50% ethanol, afterwards.

d. Analysis

i. **Samples:** *Standard Solution and Sample Solution* Apply the samples to the TLC plate and pre-dry. Develop the chromatogram in a chamber saturated for 20 min using a suitable device and remove the developed plate after drying. Examine under normal light, short wavelength UV light (254 nm), and long wavelength UV light (366 nm). Treat with derivatization reagent as instructed above. Examine again under normal light, short wavelength UV light (254 nm), and long wavelength UV light (366 nm).

ii. **Acceptance criteria:** The sequence of zones present in the chromatogram of the sample solution must exhibit similar pattern to the reference chromatogram. Other faint bands may be present in the chromatogram of the sample solution. See *Annex* for the reference chromatogram.

FOREIGN MATTER

<2> not more than 1%

LOSS ON DRYING/MOISTURE CONTENT

<3> not more than 10%

ALCOHOL (95% v/v)-SOLUBLE EXTRACTIVES

<4> not less than 21%

WATER-SOLUBLE EXTRACTIVES

<4> not less than 18%

TOTAL ASH

<5> not more than 11%

ACID-INSOLUBLE ASH

<5> not more than 3%

WATER-SOLUBLE ASH

<5> not more than 3%

TEST FOR TOXIC HEAVY METALS

<54> The toxic heavy metal limits should conform to the specified limits stated in AO 184 s. 2004. Sample should not have more than 0.3 ug/g (ppm) for arsenic and cadmium, 10 ug/g (ppm) for lead, and 0.5 ug/g (ppm) for mercury.

AFLATOXIN

<57> Total aflatoxin shall conform to the prescribed specification of 20 mcg/kg and aflatoxin B1 of 10 mcg/kg by ELISA method or liquid chromatography

PESTICIDE LIMITS

<56> shall conform to the limits defined by WHO/FAO Codex Alimentarius

MICROBIAL LIMIT

<52> The total aerobic microbial count does not exceed 10^5 cfu/g, the total combined molds and yeast count does not exceed 10^3 cfu/g, and it meets the requirements of the tests for the absence of *Salmonella* species and *Escherichia coli.* Other enterobacteria do not exceed 10^3 cfu/g.

TRADITIONAL USES

It is used as an abortifacient, laxative, for parturition, anemia, constipation, dermatitis, dyspepsia, fever, diuretic, insecticide, treatment for skin diseases (e.g. ringworm, skin sores, scabies, etc.), snake bite, larvicide, and treatment for asthma and bronchitis.

Sennae Alatae Lotion
Akapulko Lotion

DEFINITION

Sennae Alatae Folium Pulveratum processed in topical preparation. Each 5mL of Sennae Alatae Lotion contains 2.5g of dried *akapulko* leaf powder as a decoction.

WARNING

Akapulko have been reported to cause allergic reactions in sensitive people.

PACKAGING AND STORAGE

Preserve in well-closed, light-resistant containers.

LABELING

Print the official title and the national title of the herbal material on the label as directed under *General Notices and Requirements* Part I.

pH

<34> 5.2-6.5

VISCOSITY

<35> not less than 100cps.

MICROBIAL LIMIT

<52> The total aerobic microbial count does not exceed 10^2 cfu/g, the total combined molds and yeast count does not exceed 10^2 cfu/g, and it meets the requirements of the tests for the absence of *Salmonella* species and *Escherichia coli.* Other enterobacteria do not exceed 10^2 cfu/g

MEDICAL USES

Treatment for cutaneous fungal infections such as tinea versicolor.

VITICIS NEGUNDONIS

Viticis Negundonis Folium
Lagundi Leaf

DEFINITION

Viticis Negundonis Folium consists of dried leaf and flowering top of *Vitex negundo* L. (fam. Verbenaceae).

SCIENTIFIC NAME

Vitex negundo L.

SYNONYMS

Agnus-castus negundo Carrière
Vitex agnus-castus var. *negundo* (L.) Kuntze
Vitex agnus-castus var. *negundoides* Kuntze
Vitex leucoxylon Blanco
Vitex negundo f. alba C.P'ei

SELECTED COMMON NAMES

Filipino: *dabtan* (If.), *dangla* (Ilk.), *kalipapa-madam* (Mag.), *kamalan* (Tag.), *limo-limo* (Ilk.), *limpapa* (Sul.); *lingei* (Bon.), *lingo-lingo* (Ilk.), *mapugay* (Sul.) *moore-oso* (Sul.); *sugarai* (Bag.); *tugas* (S. L. Bis.); *five-leaved chaste tree* (Eng.), *Five-leaved chaste tree*, *horseshoe vitex* (En). Indonesia: *ai tuban* (Ambon), *lagundi laut laki-laki* (Malay, Moluccas). Malaysia: *lagundi*, *lemuning*, *lenggundi* (Peninsular). Papua New Guinea: *paparau* (Kurtachi, Bougainville). Philippines: *lagundi* (Filipino), *dangla* (Iloko). Cambodia: *trasiet*. Thailand: *khon thee khamoa* (central), *ku-no-kaa-mo* (Malay, Pattani), *kuuning* (Malay, Narathiwat). Vietnam: *ng[ux] tr[ar]o*.

MAJOR CHEMICAL CONSTITUENTS

Viticis Negundonis Folium yields a volatile oil-containing monoterpenes and sesquiterpenes. Flavonoids and phenylpropanoids such as artemitin, hesperitin, luteolin, vitexin, derivatives of ferulic acid and of caffeic acid, and various flavanones, flavones, flavonols, have been identified. A number of compounds have also been identified, such as iridoids (e.g., aucubin, lagundinin): diterpenes (e.g.vitexilactone, abietic acid derivatives); triterpenes and benzenoids (e.g., stilbene and its derivatives, and syringic acid). The presence of cytotoxic flavone analogues of vitexicarpin has recently been reported.

MORPHOLOGICAL DESCRIPTION OF THE PLANT

Vitex negundo is an erect, branched shrub between 2-5 m high, thriving in wastelands and thickets and widely distributed in the country. The leaf has five stalked leaflets. The inflorescence has numerous florets, mostly occurring as terminal pubescent panicles. Corolla is densely hairy in the throat with the middle lobe of the lower lip being the largest. The blue flowers, 6-7 mm long, are supported by pubescent toothed sepals. The globose fruit measuring, approximately 4 mm in diameter, is a succulent type of drupe.

MACROSCOPIC DESCRIPTION

The petiolated leaves are five-foliated, with the middle leaflets larger than the other leaflets. Each leaflet measures 4-10 cm long. The leaves are entire, lanceolate (lance-shaped), with acuminate apex and are hairy beneath. Leaves emit an aroma when crushed.

MICROSCOPIC DESCRIPTION

Transverse section of the leaf shows the upper palisade layer of the mesophyll consisting of elongated chlorenchyma cells at right angles with the leaf surface while the lower spongy layer has irregularly shaped chlorenchyma cells with large air spaces. Three to four rows of collenchyma cells can be found beneath the upper and lower surfaces of the midrib. Beneath these collenchyma cells are large spherical parenchyma cells with intercellular spaces. At the side of the midrib near the palisade area are parenchyma cells with chloroplastids (chlorenchyma). Vascular bundles have xylem surrounded by phloem. The upper and lower epidermis have anomocytic type of stomata. Trichomes densely cover the lower epidermal layer while the upper epidermis has only a few glandular and unicellular trichomes.

WARNING

May cause allergic reactions to sensitive people, who may develop itchiness, nausea, vomiting and diarrhea.

PACKAGING AND STORAGE

Preserve in well-closed, light-resistant containers, check occasionally for the presence of molds and for any evidence of insect attack.

LABELING

Print the official title and the national title of the herbal material on the label as directed under *General Notices and Requirements* Part I.

TRADITIONAL USES

Decoction is prepared to treat cough, fever, respiratory diseases, and as anti-inflammatory agent. Treatment for cold, fever, flu, chronic bronchitis, pharyngitis, rheumatism, dyspepsia, boils, diarrhea, and certain skin or digestive problems.

Viticis Negundonis Folium Pulveratum
Lagundi Leaf Powder

DEFINITION

Viticis Negundonis Folium reduced to powder form.

SCIENTIFIC NAME

Vitex negundo L.

SYNONYMS

Agnus-castus negundo Carrière
Vitex agnus-castus var. *negundo* (L.) Kuntze
Vitex agnus-castus var. *negundoides* Kuntze
Vitex leucoxylon Blanco
Vitex negundo f. alba C.P'ei

SELECTED COMMON NAMES

Filipino: *dabtan* (If.), *dangla* (Ilk.), *kalipapa-madam* (Mag.), *kamalan* (Tag.), *limo-limo* (Ilk.), *limpapa* (Sul.); *lingei* (Bon.), *lingo-lingo* (Ilk.), *mapugay* (Sul.) *moore-oso* (Sul.); *sugarai* (Bag.); *tugas* (S. L. Bis.); *five-leaved chaste tree* (Eng.), *Five-leaved chaste tree*, *horseshoe vitex* (En). Indonesia: *ai tuban* (Ambon), *lagundi laut laki-laki* (Malay, Moluccas). Malaysia: *lagundi*, *lemuning*, *lenggundi* (Peninsular). Papua New Guinea: *paparau* (Kurtachi, Bougainville). Philippines: *lagundi* (Filipino), *dangla* (Iloko). Cambodia: *trasiet*. Thailand: *khon thee khamoa* (central), *ku-no-kaa-mo* (Malay, Pattani), *kuuning* (Malay, Narathiwat). Vietnam: *ng[ux] tr[ar]o*.

MAJOR CHEMICAL CONSTITUENTS

Viticis Negtindonis Folium yields a volatile oil- containing monoterpenes and sesquiterpenes. Flavonoids and phenylpropanoids such as artemitin, hesperitin, luteolin, vitexin, derivatives of ferulic acid and of caffeic acid, and various flavanones, flavones. and flavonols have been identified. A number of compounds have also been identified, such as iridoids (e.g., aucubin, lagundinin): diterpenes (e.g.vitexilactone, abietic acid derivatives); triterpenes and benzenoids (e.g., stilbene and its derivatives, and syringic acid). The presence of cytotoxic flavone analogues of vitexicarpin has recently been reported.

WARNING

May cause allergic reactions to sensitive people, who may develop itchiness, nausea, vomiting and diarrhea.

PACKAGING AND STORAGE

Preserve in well-closed, light-resistant containers, check occasionally for the presence of molds.

LABELING

Print the official title and the national title of the herbal material on the label as directed under *General Notices and Requirements* Part I.

IDENTIFICATION

a. Standard Solution

(1) 2.0 mg/mL of Negundoside RS in methanol. Sonicate for 1 min and mix by vortex. (2) 2.0 mg/mL of 3,5-dihydroxybenzoic acid RS in methanol. Sonicate for 1 min and mix by vortex.

b. Sample Solution

Sonicate 2.5 g of *Vitex negundo* Leaf, finely powdered, in 25 mL methanol for 30 min with vortex mixing every 15 min. Filter the mixture using filter paper and use the collected filtrate.

c. Chromatographic System

i. **Adsorbent:** TLC plate silica gel 60 F254 (20x10 cm)

ii. **Application volume:** 0.2 µL sprayed as 9 mm bands using suitable device

iii. **Relative humidity:** Condition the plate to a relative humidity of about 33% for 15 min using a suitable device.

iv. **Developing solvent system:** a mixture of ethyl acetate, glacial acetic acid, and water (80:10:5)

v. **Developing distance:** 85 mm

vi. **Derivatization reagent:** Anisaldehyde-sulfuric acid reagent – Mix in order of 85 mL cold methanol, 10 mL glacial acetic acid, 0.5 mL p-anisaldehyde, and 5 mL concentrated sulfuric acid (perform in ice bath).

d. Analysis

i. **Samples:** *Standard Solution and Sample Solution* Apply the samples to the TLC plate and pre-dry. Develop the chromatogram in a chamber saturated for 20 min using a suitable device and remove the developed plate after drying. Examine under normal light, short wavelength UV light (254 nm), and long wavelength UV light (366 nm). Treat with derivatization reagent, and heat at 100 for 7 min. After cooling, examine again under normal light, short wavelength UV light (254 nm), and long wavelength UV light (366 nm).

ii. **Acceptance criteria:** The sequence of zones present in the chromatogram of the sample solution must exhibit similar pattern to the reference chromatogram. Other faint bands may be present in the chromatogram of the sample solution. See *Annex* for the reference chromatogram.

FOREIGN MATTER

<2> not more than 2%

LOSS ON DRYING/MOISTURE CONTENT

<3> not more than 10%

ALCOHOL (95% v/v)-SOLUBLE EXTRACTIVES

<4> not less than 6%

WATER-SOLUBLE EXTRACTIVES

<4> not more than 3%

TOTAL ASH

<5> not more than 9%

ACID-INSOLUBLE ASH

<5> not more than 2%

WATER-SOLUBLE ASH

<5> not more than 3%

TEST FOR TOXIC HEAVY METALS

<54> The toxic heavy metal limits should conform to the specified limits stated in AO 184 s. 2004. Sample should not have more than 0.3 ug/g (ppm) for arsenic and cadmium, 10 ug/g (ppm) for lead, and 0.5 ug/g (ppm) for mercury.

AFLATOXIN

<57> Total aflatoxin shall conform to the prescribed specification of 20 mcg/kg and aflatoxin B1 of 10 mcg/kg by ELISA method or liquid chromatography

PESTICIDE LIMITS

<56> shall conform to the limits defined by WHO/FAO Codex Alimentarius

MICROBIAL LIMIT

<52> The total aerobic microbial count does not exceed 10^5 cfu/g, the total combined molds and yeast count does not exceed 10^4 cfu/g, and it meets the requirements of the tests for the absence of *Salmonella* species and *Escherichia coli.* Other enterobacteria do not exceed 10^3 cfu/g.

TRADITIONAL USES

Decoction is prepared to treat cough, fever, respiratory diseases, and as anti-inflammatory agent. Treatment for cold, fever, flu, chronic bronchitis, pharyngitis, rheumatism, dyspepsia, boils, diarrhea, and certain skin or digestive problems.

Viticis Negundonis Tablet
Lagundi Tablet

DEFINITION

Viticis Negundonis Folium Pulveratum processed to tablet form. Each tablet may contain 300 or 600 mg of *lagundi* leaf powder.

WARNING

The possibility of adverse effects on pregnant and lactating women has not been established. Patients with allergies to Vitex negundo (lagundi) plants may develop itchiness, nausea, vomiting and diarrhea.

PACKAGING AND STORAGE

Preserve in well-closed, light-resistant containers. Store at temperature not exceeding 30˚C.

LABELING

Print the official title and the national title of the herbal material on the label as directed under *General Notices and Requirements* Part I.

UNIFORMITY OF DOSAGE UNITS

<31> meets the requirements.

DISINTEGRATION

<38> All of the tablets disintegrated completely with no palpable firm core in not more than 30 min.

FRIABILITY

<37> maximum mean weight loss from the samples is not more than 1%

MICROBIAL LIMIT

<52> The total aerobic microbial count does not exceed 10^5 cfu/g, the total combined molds and yeast count does not exceed 10^4 cfu/g, and it meets the requirements of the tests for the absence of *Salmonella* species and *Escherichia coli.* Other enterobacteria do not exceed 10^3 cfu/g.

MEDICAL USES

Vitex negundo Tablets are used in the treatment of respiratory systems such as cough of non-bacterial origin.

Viticis Negundonis Syrup
Lagundi Syrup

DEFINITION

Viticis Negundonis Folium Pulveratum processed to syrup form. For each 5 mL of syrup may contain 300 or 600 mg of *lagundi* leaf powder.

PACKAGING AND STORAGE

Preserve in well-closed, light-resistant containers. Store at temperature not exceeding 30˚C.

LABELING

Print the official title and the national title of the herbal material on the label as directed under *General Notices and Requirements* Part I.

WARNING

The possibility of adverse effects on pregnant and lactating women has not been established. Patients with allergies to *vitex negundo* may develop itchiness, nausea, vomitting and diarrhea.

pH

<34> 2.75-3.80

DELIVERABLE VOLUME

<33> meet the requirements

VISCOSITY

<35> not less than 35cps

MICROBIAL LIMIT

<52> The total aerobic microbial count does not exceed 10^5 cfu/g, the total combined molds and yeast count does not exceed 10^4 cfu/g, and it meets the requirements of the tests for the absence of *Salmonella* species and *Escherichia coli.* Other enterobacteria do not exceed 10^3 cfu/g.

MEDICAL USES

Vitex negundo syrup is used in the treatment of respiratory systems such as cough of non-bacterial origin and asthma.

ZINGIBERIS OFFICINALIS

Zingiberis Officinalis Rhizoma
Luya Rhizome

DEFINITION

Zingiberis Officinalis Rhizoma is the dried rhizome of *Zingiber officinale* Roscoe (Fam. Zingiberaceae).

SCIENTIFIC NAME

Zingiber officinale Roscoe

SYNONYMS

Amomum zingiber L.
Zingiber aromaticum Noronha
Zingiber blancoi Hassk.
Zingiber zingiber H.Karst.

SELECTED COMMON NAMES

Filipino: *agat* (Pang.), *basing* (Ilk.), *laial* (If.), *laya* (Ilk., Bon., Ibn., It.), *loya*, *paginsen* (Lan.), *luy-a* (Bis.), *naha* (Bat.), *ginger* (Eng.), *gengibre* (Spa.), *Gingembre* (Fr). Indonesia: *jahe*, *jae*, *lia*. Malaysia: *haliya*, *jahi*, *atuja*. Papua New Guinea: *kawawar*, *kawawari*. Philippines: *luya* (Tagalog), *baseng* (Ilocano), *laya* (Bicol). *Burma* (Myanmar): *gyin*. Cambodia: *khnhei*, *khnhei phlung*. Laos: *khi:ng*. Thailand: *khing*, *khing-daeng*. Vietnam: *g[uwf]ng*, *sinh kh[uw][ow]ng*.

MAJOR CHEMICAL CONSTITUENTS

Zingiberis Officinalis Rhizoma contains phenolic and terpene compounds that are responsible for its strong antioxidant and anti-inflammatory activity. Major polyphenolic compounds found in ginger rhizomes are gingerols, namely 4-gingerol, 6-gingerol, 8-gingerol, 10-gingerol, and these are thermolabile compounds that can be converted into shogaols upon dehydration. Other phenolic compounds found in ginger rhizomes are quercetin, zingiberene, and gingerenone-A. The terpene components of Zingiberis officinalis Rhizoma that constitutes the majority of its essential oil includes -bisabolene, -curcumene, zingiberine, -farnesene, and -sesquiphellandrene.

MORPHOLOGICAL DESCRIPTION OF THE PLANT

The leafy stem is usually erect, arising from a fleshy underground aromatic rootstock/rhizome. The sessile leaves range from linear to lanceolate in shape.

MACROSCOPIC DESCRIPTION

When dried, the light brown underground rhizome is laterally compressed, with longitudinal striations and annulated nodes, branches irregular, with remnants of scale leaves. The almost sessile, distichously attached leaves are lanceolate to linear lanceolate and elliptic. This plant has an ovoid to ellipsoid spike inflorescence arising directly from the rhizome. The scape (a peduncle rising from the ground or near it, bearing a few flowers) emerges directly from the rootstock. Greenish yellow to yellow corolla tubes with an oblong to obovate slightly purplish lip, are few and are surrounded by pale green ovate bracts. Ovary is inferior and trilocular. The capsule fruit has black seeds enveloped by fleshy outgrowths or arils from the base.

MICROSCOPIC DESCRIPTION

Transverse section of the rhizome reveals thin-walled yellowish brown cells in the single-layered epidermis, a narrow parenchymatous hypodermis, and a broad central stele of parenchymatous cells. Abundant flattened ovate starch grains, oil cells with suberized walls, and greenish yellow content abound in the ground tissue. Dark reddish brown pigmented cells are also found singly or in rows in the ground tissue as well as in the accompanying vascular bundles. Between the cortical parenchyma and the stele are the pale brown thin walled endodermal cells with suberin. In the scattered collateral vascular bundles, the vessels have spiral or netted wall thickenings.

WARNING

May lead to contact dermatitis for sensitive individuals.

PACKAGING AND STORAGE

Preserve in well-closed, light-resistant containers, check occasionally for any evidence of insect attack.

LABELING

Print the official title and the national title of the herbal material on the label as directed under *General Notices and Requirements* Part I.

TRADITIONAL USES

Traditionally used as a flavoring agent, as anti-oxidant, anti-inflammatory agent, antinausea, and anticancer. Also use for the treatment of colds, nausea, arthritis, migraines, and hypertension. Traditionally used against diabetes, catarrh, asthma, rheumatism, stroke, gingivitis, toothache, constipation, and nervous system disease.

Zingiberis Officinalis Rhizoma Pulveratum
Luya Rhizome Powder

DEFINITION

Zingiberis Officinalis Rhizoma reduced to powder form.

SCIENTIFIC NAME

Zingiber officinale Roscoe

SYNONYMS

Amomum zingiber L.
Amomum zinziba Hill
Zingiber cholmondeleyi (F.M. Bailey) K. Schum.
Zingiber missionis Wall.

SELECTED COMMON NAMES

Filipino: *agat* (Pang.), *basing* (Ilk.), *laial* (If.), *laya* (Ilk., Bon., Ibn., It.), *loya*, *paginsen* (Lan.), *luy-a* (Bis.), *naha* (Bat.), *ginger* (Eng.), *gengibre* (Spa.), *Gingembre* (Fr). Indonesia: *jahe*, *jae*, *lia*. Malaysia: *haliya*, *jahi*, *atuja*. Papua New Guinea: *kawawar*, *kawawari*. Philippines: *luya* (Tagalog), *baseng* (Ilocano), *laya* (Bicol). *Burma* (Myanmar): *gyin*. Cambodia: *khnhei*, *khnhei phlung*. Laos: *khi:ng*. Thailand: *khing*, *khing-daeng*. Vietnam: *g[uwf]ng*, *sinh kh[uw][ow]ng*.

MAJOR CHEMICAL CONSTITUENTS

Zingiberis Officinalis Rhizoma contains phenolic and terpene compounds that are responsible for its strong antioxidant and anti-inflammatory activity. Major polyphenolic compounds found in ginger rhizomes are gingerols, namely 4-gingerol, 6-gingerol, 8-gingerol, 10-gingerol, and these are thermolabile compounds that can be converted into shogaols upon dehydration. Other phenolic compounds found in ginger rhizomes are quercetin, zingiberene, and gingerenone-A. The terpene components of Zingiberis officinalis Rhizoma that constitutes the majority of its essential oil includes -bisabolene, -curcumene, zingiberine,-farnesene, and -sesquiphellandrene.

WARNING

May lead to contact dermatitis for sensitive individuals.

PACKAGING AND STORAGE

Preserve in well-closed, light-resistant containers.

LABELING

Print the official title and the national title of the herbal material on the label as directed under *General Notices* and *Requirements* Part I.

IDENTIFICATION

a. Standard Solution

1.0 mg/mL of Gingerol RS in methanol. Sonicate for 1 min. and mix by vortex.

b. Sample Solution

Sonicate 2.5 g of *Zingiberis officinalis Rhizome*, finely powdered, in 25 mL methanol for 30 min with vortex mixing every 15 min. Filter the mixture using filter paper and use the collected filtrate.

c. Chromatographic System

i. **Adsorbent:** TLC plate silica gel 60 F254 (20x10 cm)

ii. **Application volume:** 0.2 µL sprayed as 9 mm bands using suitable device.

iii. **Relative humidity:** Condition the plate to a relative humidity of about 33% for 20 min using a suitable device.

iv. **Developing solvent system:** mixture of hexane, ethyl acetate, formic acid (62.19:37.31:0.5).

v. **Developing distance:** 85 mm

vi. **Derivatization reagent:** Anisaldehyde-sulfuric acid reagent – Mix in order of 85 mL cold methanol, 10 mL glacial acetic acid, 0.5 mL p-anisaldehyde, and 5 mL concentrated sulfuric acid (perform in ice bath).

d. Analysis

i. **Samples:** *Standard Solution and Sample Solution* Apply the samples to the TLC plate and pre-dry. Develop the chromatogram in a chamber saturated for 30 min using a suitable device and remove the developed plate after drying. Examine under normal light, short wavelength UV light (254 nm), and long wavelength UV light (366 nm). Treat with derivatization reagent, and dry at 100°C for 10 mins. Examine again under normal light, short wavelength UV light (254 nm), and long wavelength UV light (366 nm).

ii. **Acceptance criteria:** The sequence of zones present in the chromatogram of the sample solution must exhibit similar pattern to the reference chromatogram. Other faint bands may be present in the chromatogram of the sample solution. See *Annex* for the reference chromatogram.

FOREIGN MATTER

<2> not more than 2%

LOSS ON DRYING/MOISTURE CONTENT

<3> not more than 10%

ALCOHOL (95% v/v)-SOLUBLE EXTRACTIVES

<4> not less than 7%

WATER-SOLUBLE EXTRACTIVES

<4> not less than 13%

TOTAL ASH

<5> not more than 13%

ACID-INSOLUBLE ASH

<5> not more than 3%

WATER-SOLUBLE ASH

<5> not more than 6%

TEST FOR TOXIC HEAVY METALS

<54> The toxic heavy metal limits should conform to the specified limits stated in AO 184 s. 2004. Sample should not have more than 0.3 ug/g (ppm) for arsenic and cadmium, 10 ug/g (ppm) for lead, and 0.5 ug/g (ppm) for mercury.

AFLATOXIN

<57> Total aflatoxin shall conform to the prescribed specification of 20 mcg/kg and aflatoxin B1 of 10 mcg/kg by ELISA method or liquid chromatography

PESTICIDE LIMITS

<56> shall conform to the limits defined by WHO/FAO Codex Alimentarius

MICROBIAL LIMIT

<52> The total aerobic microbial count does not exceed 10^5 cfu/g, the total combined molds and yeast count does not exceed 10^3 cfu/g, and it meets the requirements of the tests for the absence of *Salmonella* species and *Escherichia coli.* Other enterobacteria do not exceed 10^3 cfu/g.

TRADITIONAL USES

Traditionally used as a flavoring agent, as anti-oxidant, anti-inflammatory agent, antinausea, and anticancer. Also use for the treatment of colds, nausea, arthritis, migraines, and hypertension. Traditionally used against diabetes, catarrh, asthma, rheumatism, stroke, gingivitis, toothache, constipation, and nervous system disease.

Part II. Medicinal Plants

ALLII SATIVI

Allii Sativi Bulbus
Bawang Cloves

DEFINITION

Allii Sativi Bulbus is the dried compound bulbs of *Allium sativum* L. 'Ilocos White' (Fam. Liliaceae/Alliaceae).

SCIENTIFIC NAME

Allium sativum L.

SYNONYMS

Allium controversum Schrad. ex Willd.
Allium longiscupis Regel
Allium ophioscorodon Link
Allium pekinense Prokh.

SELECTED COMMON NAMES

ahos (Bis.), *ahus* (Ibn.), *bawang* (Tag., Ilk.), *hisiao suan* (Chi.), garlic (Eng.), *ajos* (Spa.), *bawang putih* (Indo., Mal.), *khtüm sââ* (Cam.), *kath'ièm* (Laos), *krathiam* (Thai)

MAJOR CHEMICAL CONSTITUENTS

Allium sativum bulbus is abundant in sulfur-containing compounds. Non-volatile thiosulfinates such as alliin and its derivatives are attributed with the major pharmacological activity exhibited by garlic. Volatile sulfur-containing compounds like allicin, ajoenes, vinyldithiins, and sulfides like diallyl disulfide, diallyl trisulfide are also observed to have potent bioactive properties that contribute to the medicinal value of garlic bulbs.

MORPHOLOGICAL DESCRIPTION OF THE PLANT

Allium sativum is a low herb reaching only 30-60 cm high, with its prominent narrow long pointed leaves and a much reduced stem. The flattened leaves are narrow, linear or elongated. A cylindrical scape serves as the peduncle rising from the ground or near it and extends beyond the leaves. This bears a cluster of white florets formed into a globose umbel type of inflorescence, subtended by long, thin, dry, membranous bracts. Stamens are not exerted from the perianth. Sepals are oblong-shaped, greenish white tinged with purple.

MACROSCOPIC DESCRIPTION

The reduced stem of *Allium sativum* gives rise to a broadly ovoid underground bulb which is divided into several closely crowded angular, truncated tubers (cloves). The bulb of *Allium sativum* has each clove measuring 5-10 mm in diameter. A silky white to pinkish membranous skin encloses the dense cluster of cloves/bulblets. The clove has a strong odor with a persistently pungent and acrid taste.

MICROSCOPIC DESCRIPTION

Each clove of a bulb is made up of outer scales, a uniseriate epidermal layer enclosing a mesophyll without any chlorophyll. Vascular bundles can be found in several layers of large parenchymatous cells beneath the epidermal layer. Two to three layers of rectangular cells with broadly angular end walls, comprise the dry scales. Cells contain numerous calcium oxalate crystals, specifically rhomboidal.

WARNING

Frequent contact may result in allergic reactions such as dermatitis and asthma.

PACKAGING AND STORAGE

Preserve in well-closed, light-resistant containers in a cool, dry place.

LABELING

Print the official title and the national title of the herbal material on the label as directed under *General Notices and Requirements* Part I.

TRADITIONAL USES

It is the universal folk spice and food, remedy against typhus, dysentery, cholera, influenza. It is used in Indian medicine as a tonic, roborans, to cure lack of appetite, weakness, cough, skin disease, rheumatism, hemorrhoids, etc. Traditionally used to prevent cardiovascular diseases, regulating blood pressure, lowering blood sugar and cholesterol levels, effective as anti-bacterial, anti-fungal, and anti-parasitic, and having antitumoral and antioxidant property.

Allii Sativi Bulbus Pulveratum
Bawang Cloves Powder

DEFINITION

Allii Sativi Bulbus that has been cut, oven-dried at a temperature not exceeding 60°C and reduced to powder form.

SCIENTIFIC NAME

Allium sativum L.

SYNONYMS

Allium controversum Schrad. ex Willd.
Allium longiscupis Regel
Allium ophioscorodon Link
Allium pekinense Prokh.

SELECTED COMMON NAMES

ahos (Bis.), *ahus* (Ibn.), *bawang* (Tag., Ilk.), *hisiao suan* (Chi.), garlic (Eng.), *ajos* (Spa.), *bawang putih* (Indo., Mal.), *khtüm sââ* (Cam.), *kath'ièm* (Laos), *krathiam* (Thai)

MAJOR CHEMICAL CONSTITUENTS

Allium sativum bulbus is abundant in sulfur-containing compounds. Non-volatile thiosulfinates such as alliin and its derivatives are attributed with the major pharmacological activity exhibited by garlic. Volatile sulfur-containing compounds like allicin, ajoenes, vinyldithiins, and sulfides like diallyl disulfide, diallyl trisulfide are also observed to have potent bioactive properties that contribute to the medicinal value of garlic bulbs.

WARNING

Frequent contact may result in allergic reactions such as dermatitis and asthma.

PACKAGING AND STORAGE

Preserve in well-closed, light-resistant containers in a cool, dry place.

LABELING

Print the official title and the national title of the herbal material on the label as directed under *General Notices and Requirements* Part I.

IDENTIFICATION

a. Standard Solution

1.0 mg/mL of Alliin USP in methanol. Sonicate for 1 min and mix by vortex.

b. Sample Solution

Sonicate 2.5 g of *Allium sativum* Cloves, finely powdered, in 25 mL methanol for 30 min with vortex mixing every 5 min. Filter the mixture using filter paper and use the collected filtrate.

c. Chromatographic System

i. **Adsorbent:** TLC plate silica gel 60 F254 (20x10 cm)

ii. **Application volume:** 0.2 µL sprayed as 9 mm bands using suitable device.

iii. **Relative humidity:** Condition the plate to a relative humidity of about 33% for 20 min using a suitable device.

iv. **Developing solvent system:** a mixture of butanol, acetic acid, water, chloroform (47.63:22.13:19.83:10.42)

v. **Developing distance:** 85 mm

vi. **Derivatization reagent:** Ninhydrin spray reagent – Dissolve 200 mg of ninhydrin in 100 mL water.

d. **Analysis**

i. **Samples:** *Standard Solution and Sample Solution* Apply the samples to the TLC plate and pre-dry. Develop the chromatogram in a chamber saturated for 30 min using a suitable device and remove the developed plate after drying. Examine under normal light, short wavelength UV light (254 nm), and long wavelength UV light (366 nm). Treat with derivatization reagent, and dry at 100°C for 10 mins. Examine again under normal light, short wavelength UV light (254 nm), and long wavelength UV light (366 nm).

ii. **Acceptance criteria:** The sequence of zones present in the chromatogram of the sample solution must exhibit similar pattern to the reference chromatogram. Other faint bands may be present in the chromatogram of the sample solution. See *Annex* for the reference chromatogram.

FOREIGN MATTER

<2> not more than 1%

LOSS ON DRYING/MOISTURE CONTENT

<3> not more than 10%

ALCOHOL (95% v/v)-SOLUBLE EXTRACTIVES

<4> not less than 5%

WATER-SOLUBLE EXTRACTIVES

<4> not less than 40%

TOTAL ASH

<5> not more than 8%

ACID-INSOLUBLE ASH

<5> not more than 1%

WATER-SOLUBLE ASH

<5> not more than 6%

TEST FOR TOXIC HEAVY METALS

<54> The toxic heavy metal limits should conform to the specified limits stated in AO 184 s. 2004. Sample should not have more than 0.3 ug/g (ppm) for arsenic and cadmium, 10 ug/g (ppm) for lead, and 0.5 ug/g (ppm) for mercury

AFLATOXIN

<57> Total aflatoxin shall conform to the prescribed specification of 20 mcg/kg and aflatoxin B1 of 10 mcg/kg by ELISA method or liquid chromatography

PESTICIDE LIMITS

<56> shall conform to the limits defined by WHO/FAO Codex Alimentarius

MICROBIAL LIMIT

<52> The total aerobic microbial count does not exceed 10^5 cfu/g, the total combined molds and yeast count does not exceed 10^3 cfu/g, and it meets the requirements of the tests for the absence of *Salmonella* species and *Escherichia coli.* Other enterobacteria do not exceed 10^3 cfu/g.

TRADITIONAL USES

It is the universal folk spice and food, remedy against typhus, dysentery, cholera, influenza. It is used in Indian medicine as a tonic, roborans, to cure lack of appetite, weakness, cough, skin disease, rheumatism, haemorrgoids, etc. Traditionally used to prevent cardiovascular diseases, regulating blood pressure, lowering blood sugar and cholesterol levels, effective as anti-bacterial, anti-fungal, and anti-parasitic, and having antitumoral and antioxidant property.

CASSIAE FISTULAE

Cassiae Fistulae Folium
Kanya Pistula Leaf

DEFINITION

Cassia Fistula Folium is the dried leaf of *Cassia fistula* L. (Fam. Fabaceae).

SCIENTIFIC NAME

Cassia fistula L.

SYNONYMS

Bactyrilobium fistula Willd.
Cassia bonplandiana DC.
Cassia excelsa Kunth
Cassia fistuloides Collad.
Cathartocarpus fistula Pers.

SELECTED COMMON NAMES

Filipino: *bitsula* (C. Bis.), *fistula* (C. Bis., Tag.), *ibabau* (Bis.), *lapadlapad* (Tagb.), *lambayong* (Bis.); *golden shower*, *India laburnum*, *pudding pipe tree*, *purging cassia* (Eng.), *caña fistula* (Spa.), *Golden shower*, *Indian laburnum* (En). *Caneficier* (Fr). Indonesia: *trengguli* (Javanese), *bobondelan* (Sundanese), *klobop* (Madurese). Malaysia: *bereksa*, *tengguli*, *rajah kayu*. Cambodia: *reach*, *reach speu*, *reach chhpoeus*. Laos: *khoun* (general). Thailand: *khuun* (central, northern), *lom laeng* (northern), *ratchaphruek* (central). Vietnam: *c[aa]y b[of] c[aj]p n[uw] [ows]c*, *mu[oof]ng ho[af]ng y[ees]n*.

MAJOR CHEMICAL CONSTITUENTS

Cassia Fistula Folium contains anthraquinone glycosides like rhein, chrysophanol, and physcion. Compounds such as ()-epiafzelechin 3-O-B-D-glucopyranoside, 7 biflavonoids and two triflavonoids together with () epiafzelechin, ()-epicatechin and procyanidin B-2 were identified from the leaves of kanya fistula as well as -sitosterol, sennoside A and sennoside B.

MORPHOLOGICAL DESCRIPTION OF THE PLANT

Cassia fistula (popularly known as the golden shower) is an introduced Tropical Asian moderate-sized deciduous tree that annually sheds its leaves. Belonging to family Fabaceae, it is easily recognizable by its eyecatching pendulous showy bright yellow flowers. These yellow florets are borne in long pendulous racemous inflorescence stalks covering the slightly drooping branches. Sepals, 6-8 mm long, are smooth and deciduous. The veined petals are obovate, 18-25 mm long, bright yellow with a short claw at the base. The stamens are two ranked (stamens grouped into upper and lower stamens, with lower stamens having longer filaments). Fruit is a cylindric pod, that is pendulous, smooth, shining and dark brown. Numerous small, ovoid, slightly compressed, smooth, shining and yellowish brown seeds are embedded in black sweet pulp which are separated by a thin transverse septum.

MACROSCOPIC DESCRIPTION

Leaves have alternate leaf attachment are evenly pinnately compound, smooth and measure 30-40 cm long. Leaf margin entire (leaf margins smooth, continuous, neither toothed nor notched or divided), undulating (leaf margins wavy). The elliptic/ovate leaflets have an average length of 10 cm.

MICROSCOPIC DESCRIPTION

The leaf blade of *Cassia fistula* in cross section reveals a uniseriated (one layer) type of epidermis, in both the upper and lower epidermal layers. The upper epidermis (where cells assume a prominent oval form different from the usual rectangular leaf epidermal cells found in other plants), is lined with a very distinct cuticle. Epidermal cells have have numerous prismatic crystals. The palisade layer, with numerous chloropasts and prismatic crystals as well, lie beneath the upper epidermis. The loosely packed spongy layer found above the lower

epidermis has numerous chloroplasts. Lateral veins with helical type of tracheary element interspersed between the spongy cells. Some spongy cells have circular chloroplasts. Stomates are only found in the lower epidermis (i.e. hypostomatic type of stomata).

In the midrib area, cells adjacent to the upper and lower epidermis are mostly parenchymatous with isolated slightly thickened walled cells. A very distinct ring of sclerenchyma cells encircle the vascular tissues of xylem and phloem that are located close to the center of the midrib. A pith-like region made of parenchymatous cells occupy the center part of the midrib.

WARNING

Exposure may trigger heavy purgation.

PACKAGING AND STORAGE

Preserve in well-closed, light-resistant containers.

LABELING

Print the official title and the national title of the herbal material on the label as directed under *General Notices and Requirements* Part I.

TRADITIONAL USES

Leaf and fruit pulp decoction is used as a purgative. It is used to treat ringworm, and fungal skin infections. Traditionally used as purgative and against skin diseases, liver problems, hematemesis, pruritis, leucoderm, and diabetes.

Cassiae Fistulae Folium Pulveratum
Kanya Pistula Leaf Powder

DEFINITION

Cassia Fistula Folium reduced to powder form.

SCIENTIFIC NAME

Cassia fistula L.

SYNONYMS

Bactyrilobium fistula Willd.
Cassia bonplandiana DC.
Cassia excelsa Kunth
Cassia fistuloides Collad.
Cathartocarpus fistula Pers.

SELECTED COMMON NAMES

Filipino: *bitsula* (C. Bis.), *fistula* (C. Bis., Tag.), *ibabau* (Bis.), *lapadlapad* (Tagb.), *lambayong* (Bis.); *golden shower*, *India laburnum*, *pudding pipe tree*, *purging cassia* (Eng.), *caña fistula* (Spa.), *Golden shower*, *Indian laburnum* (En). *Caneficier* (Fr). Indonesia: *trengguli* (Javanese), *bobondelan* (Sundanese), *klobop* (Madurese). Malaysia: *bereksa*, *tengguli*, *rajah kayu*. Cambodia: *reach*, *reach speu*, *reach chhpoeus*. Laos: *khoun* (general). Thailand: *khuun* (central, northern), *lom laeng* (northern), *ratchaphruek* (central). Vietnam: *c[aa]y b[of] c[aj]p n[uw] [ows]c*, *mu[oof]ng ho[af]ng y[ees]n*.

MAJOR CHEMICAL CONSTITUENTS

Cassia Fistula Folium contains anthraquinone glycosides like rhein, chrysophanol, and physcion. Compounds such as ()-epiafzelechin 3-O-B-D-glucopyranoside, 7 biflavonoids and two triflavonoids together with () epiafzelechin, ()-epicatechin and procyanidin B-2 were identified from the leaves of kanya fistula as well as -sitosterol, sennoside A and sennoside B.

WARNING

Exposure may trigger heavy purgation.

PACKAGING AND STORAGE

Preserve in well-closed, light-resistant containers.

LABELING

Print the official title and the national title of the herbal material on the label as directed under *General Notices and Requirements* Part I.

IDENTIFICATION

a. Standard Solution

1 mg/mL of B-sitosterol in methanol. Sonicate until the solute is completely dissolved.

b. Sample Solution

Sonicate 2.5 g of *Cassia fistula* Leaf, finely powdered,

in 25 mL methanol for 30 min at 40° C and mix by vortex every 15 min. Filter the mixture using filter paper and use the collected filtrate.

c. **Chromatographic System**

i. **Adsorbent:** TLC plate silica gel 60 F254 (20x10 cm)

ii. **Application volume:** 0.2 µL sprayed as 9 mm bands using suitable device.

iii. **Relative humidity:** Condition the plate to a relative humidity of about 33% for 20 min using a suitable device.

iv. **Developing solvent system:** A mixture of hexane, ethyl acetate, formic acid (66.45:33.22:0.33).

v. **Developing distance:** 85 mm

vi. **Derivatization reagent:** Anisaldehyde-sulfuric acid reagent – Mix in order of 85 mL cold methanol, 10 mL glacial acetic acid, 0.5 mL p-anisaldehyde, and 5 mL concentrated sulfuric acid (perform in ice bath).

d. **Analysis**

i. **Samples:** *Standard Solution and Sample Solution* Apply the samples to the TLC plate and pre-dry. Develop the chromatogram in a chamber saturated for 30 min using a suitable device and remove the developed plate after drying. Examine under normal light, short wavelength UV light (254 nm), and long wavelength UV light (366 nm). Treat with derivatization reagent, and dry at 100°C for 10 mins. Examine again under normal light, short wavelength UV light (254 nm), and long wavelength UV light (366 nm).

ii. **Acceptance criteria:** The sequence of zones present in the chromatogram of the sample solution must exhibit similar pattern to the reference chromatogram. Other faint bands may be present in the chromatogram of the sample solution. See *Annex* for the reference chromatogram.

FOREIGN MATTER

<2> not more than 2%

LOSS ON DRYING/MOISTURE CONTENT

<3> not more than 10%

ALCOHOL (95% v/v)-SOLUBLE EXTRACTIVES

<4> not less than 6%

WATER-SOLUBLE EXTRACTIVES

<4> not less than 12%

TOTAL ASH

<5> not more than 10%

ACID-INSOLUBLE ASH

<5> not more than 2%

WATER-SOLUBLE ASH

<5> not more than 3%

TEST FOR TOXIC HEAVY METALS

<54> The toxic heavy metal limits should conform to the specified limits stated in AO 184 s. 2004. Sample should not have more than 0.3 ug/g (ppm) for arsenic and cadmium, 10 ug/g (ppm) for lead, and 0.5 ug/g (ppm) for mercury.

AFLATOXIN

<57> Total aflatoxin shall conform to the prescribed specification of 20 mcg/kg and aflatoxin B1 of 10 mcg/kg by ELISA method or liquid chromatography

PESTICIDE LIMITS

<56> shall conform to the limits defined by WHO/FAO Codex Alimentarius

MICROBIAL LIMIT

<52> The total aerobic microbial count does not exceed 10^5 cfu/g, the total combined molds and yeast count does not exceed 10^3 cfu/g, and it meets the requirements of the tests for the absence of *Salmonella* species and *Escherichia coli.* Other enterobacteria do not exceed 10^3 cfu/g.

TRADITIONAL USES

Leaf and fruit pulp decoction is used as a purgative. It is used to treat ringworm, and fungal skin infections. Traditionally used as purgative and against skin diseases, liver problems, hematemesis, pruritis, leucoderm, and diabetes.

CURCUMAE LONGA

Curcumae Longae Rhizoma
Luyang Dilaw Rhizome

DEFINITION

Curcumae Longae Rhizoma is the dried rhizome of *Curcuma longa* L. (Fam. Zingiberaceae).

SCIENTIFIC NAME

Curcuma longa L.

SYNONYMS

Amomum curcuma Jacq.
Curcuma domestica Valeton
Curcuma tinctoria Guibourt
Stissera curcuma Giseke

SELECTED COMMON NAMES

Filipino: *angai* (Pamp.), *duao*, *duwaw* (Ak., Buk., Ilk., Ley., Mis., N. Ec., Sam., Sur.), *dulao* (Cap., Dav., S. Cot., Sul., Taw., Zambo.), *gili gili* (Mag.), *hama'a* (Bat.), *kalabaga* (Bis.), *kalauag* (Mbo., Bis.), *kalawag* (Ant., Lan., Mag., Mas., Sor., S. Kud.), *kinamboi* (Bis.), *kulalo*, *pangas* (Pamp.), *kulyaw* (Ilk.), *kunig*, *kunik*, *kuning* (Ilk.), *lampuyang* (P. Bis.), *lauag* (Sub.), *parak* (Kuy.), *salampauyan* (Bag.); *long turmeric*, *round turmeric*, *turmeric* (Eng.), *Turmeric* (En). *Curcuma* (Fr). Indonesia: *kunyit* (Indonesian), *kunir* (Javanese), *koneng* (Sundanese). Malaysia: *kunyit* (Malay), *temu kunyit* (Malay), *tius* (Semang). Papua New Guinea: *kawawara*. Philippines: *dilaw* (Tagalog), *duwaw* (Cebuano), *kalawag* (Ilocano). Burma (Myanmar): *nanwin*. Cambodia: *rômiêt*, *lômiêt*. Laos: *khminz khünz*. Thailand: *khamin* (general), *khamin-kaeng* (northern), *khamin-chan* (central). Vietnam: *ngh[eej]*, *u[aas]t kim*

MAJOR CHEMICAL CONSTITUENTS

The main active components of Curcumae Longae rhizoma are curcuminoids which includes curcumin and its derivatives, namely, demethoxycurcumin, bis-demethoxycurcumin, 5'-methoxycurcurmin, dihydrocurcumin, and cyclocurcumin. Aside from curcuminoids, sesquiterpenes, steroids, and anthraquinone are also found in the extracts of *Curcuma longa* rhizome. Among the sesquiterpenes identified are germacrone, turmerone, ar-(3), α, β-turmerones, β-bisabolene, α-curcumene, zingiberene, β-sesquiphellandene, bisacurone, curcumenone, dehydrocurdinone, procurcumadiol, bisacumol, curcumenol, isoprocurcumenol, epiprocurecumenol, zedoaronediol, curlone, turmeronols A and B. Stigmasterol and β-sitosterol are some of the steroids found in the rhizomes while the antraquinone reported is 2-hydroxymethyl anthraquinone. In addition to that, compounds detected from the essential oils of *Curcuma longa* rhizome are αphellandrene, sabinene, cineol, borneol, with ar-Turmerone, α-turmerone, and β-turmerone as major constituents of the essential oil.

MORPHOLOGICAL DESCRIPTION OF THE PLANT

Curcuma longa is an erect herbaceous plant attaining a 1-1.5 m height. Broad oblong-shaped leaves, arising in two rows from the rootstock, are supported by petioles which are as long as those of the blade. The upper side of the leaves is green but underneath these is pale green. Rootstock is round to cylindrical and bright yellow inside. The peduncle supporting the spike inflorescence is borne within the clustered leaves. Flowers are pale yellow surrounded by pale green bracts but both have similar lengths.

MACROSCOPIC DESCRIPTION

Several succulent corms arise from each side of the rootstock. Roots appear as relatively stout with their endings swollen into spindles and elongated egg-shaped structures.

MICROSCOPIC DESCRIPTION

The rhizome is generally rounded in shape. Transverse section of the rhizome reveals the epidermis as rectangular or cuboidal cells of various dimensions. This serves as the outermost layer of cells enclosing the other structures. Only a few or no epidermal hairs are present. In matured rhizomes, a cork cambium is developed from the subepidermal layers beneath the epidermis. The epidermis is still retained though a cork has developed. Numerous starch grains can be found in the cortical cells and in the central region. Curcumin cells (containing yellowish colored substance) and spherical oil cells (containing orange yellow globules) were also observed in the cortical area. Cortical vascular bundles are scattered forming a discontinuous ring adjacent to the endodermis. The endodermis serves as the innermost layer of cells of the cortical region located beside the stele. Vascular bundles are also scattered within the central region.

WARNING

May trigger stomach upset, nausea, dizziness, or diarrhea.

PACKAGING AND STORAGE

Preserve in well-closed, light-resistant containers. Protect from moisture.

LABELING

Print the official title and the national title of the herbal material on the label as directed under *General Notices and Requirements* Part I.

TRADITIONAL USES

Traditionally used as a spice and coloring agent. It is used to treat chronic inflammatory diseases (e.g. chronic obstructive airway disease, asthma), and rheumatoid arthritis, chronic anterior uveitis, conjunctivitis, chicken pox, wound healing, UTI, and gall bladder and liver ailments. Also used for ulcerative colitis and irritable bowel syndrome.

Curcumae Longae Rhizoma Pulveratum

Luyang Dilaw Rhizome Powder

DEFINITION

Curcumae Longae Rhizoma reduced to powder form.

SCIENTIFIC NAME

Curcuma longa L.

SYNONYMS

Amomum curcuma Jacq.
Curcuma domestica Valeton
Curcuma tinctoria Guibourt
Stissera curcuma Giseke

SELECTED COMMON NAMES

Filipino: *angai* (Pamp.), *duao*, *duwaw* (Ak., Buk., Ilk., Ley., Mis., N. Ec., Sam., Sur.), *dulao* (Cap., Dav., S. Cot., Sul., Taw., Zambo.), *gili gili* (Mag.), *hama'a* (Bat.), *kalabaga* (Bis.), *kalauag* (Mbo., Bis.), *kalawag* (Ant., Lan., Mag., Mas., Sor., S. Kud.), *kinamboi* (Bis.), *kulalo*, *pangas* (Pamp.), *kulyaw* (Ilk.), *kunig*, *kunik*, *kuning* (Ilk.), *lampuyang* (P. Bis.), *lauag* (Sub.), *parak* (Kuy.), *salampauyan* (Bag.); *long turmeric*, *round turmeric*, *turmeric* (Eng.), *Turmeric* (En). *Curcuma* (Fr). Indonesia: *kunyit* (Indonesian), *kunir* (Javanese), *koneng* (Sundanese). Malaysia: *kunyit* (Malay), *temu kunyit* (Malay), *tius* (Semang). Papua New Guinea: *kawawara*. Philippines: *dilaw* (Tagalog), *duwaw* (Cebuano), *kalawag* (Ilocano). Burma (Myanmar): *nanwin*. Cambodia: *rômiêt*, *lômiêt*. Laos: *khminz khünz*. Thailand: *khamin* (general), *khamin-kaeng* (northern), *khamin-chan* (central). Vietnam: *ngh[eej]*, *u[aas]t kim*

MAJOR CHEMICAL CONSTITUENTS

The main active components of Curcumae Longae Rhizoma are curcuminoids which includes curcumin and its derivatives, namely, demethoxycurcumin, bis-demethoxycurcumin, 5'-methoxycurcurmin, dihydrocurcumin, and cyclocurcumin. Aside from curcuminoids, sesquiterpenes, steroids, and anthraquinone are also found in the extracts of *Curcuma longa* rhizome. Among the sesquiterpenes identified are germacrone, turmerone, ar-(3), α, β-turmerones, β-bisabolene, α-curcumene, zingiberene, β-sesquiphellandene, bisacurone, curcumenone,

dehydrocurdinone, procurcumadiol, bisacumol, curcumenol, isoprocurcumenol, epiprocurecumenol, zedoaronediol, curlone, turmeronols A and B. Stigmasterol and β-sitosterol are some of the steroids found in the rhizomes while the antraquinone reported is 2-hydroxymethyl anthraquinone. In addition to that, compounds detected from the essential oils of *Curcuma longa* rhizome are αphellandrene, sabinene, cineol, borneol, with ar-Turmerone, α-turmerone, and β-turmerone as its major constituents.

WARNING

May trigger stomach upset, nausea, dizziness, or diarrhea. Do not take medicinal amounts if pregnant. There is not enough information to rate the safety during breastfeeding.

PACKAGING AND STORAGE

Preserve in well-closed, light-resistant containers. Protect from moisture.

LABELING

Print the official title and the national title of the herbal material on the label as directed under *General Notices and Requirements* Part I.

IDENTIFICATION

a. Standard Solution

1 mg/mL of curcumin in methanol. Sonicate until the solute is completely dissolved.

b. Sample Solution

Sonicate 2.5 g of *Curcuma longa* Rhizome, finely powdered, in 25 mL methanol for 30 min and mix by vortex every 15 min. Filter the mixture using filter paper and use the collected filtrate.

c. Chromatographic System

i. **Adsorbent:** TLC plate silica gel 60 F254 (20x10 cm)

ii. **Application volume:** 0.2 µL sprayed as 9 mm bands using suitable device

iii. **Relative humidity:** Condition the plate to a relative humidity of about 33% for 20 min using a suitable device.

iv. **Developing solvent system:** a mixture of chloroform, hexane, acetone, and formic acid (69.82:19.95:9.98:0.25).

v. **Developing distance:** 85 mm

vi. **Derivatization reagent:** Anisaldehyde-sulfuric acid reagent – Mix in order of 85 mL cold methanol, 10 mL glacial acetic acid, 0.5 mL p-anisaldehyde, and 5 mL concentrated sulfuric acid (perform in ice bath).

d. Analysis

i. **Samples:** *Standard Solution and Sample Solution* Apply the samples to the TLC plate and pre-dry. Develop the chromatogram in a chamber saturated for 30 min using a suitable device and remove the developed plate after drying. Examine under normal light, short wavelength UV light (254 nm), and long wavelength UV light (366 nm). Treat with derivatization reagent, and dry at 100°C for 10 mins. Examine again under normal light, short wavelength UV light (254 nm), and long wavelength UV light (366 nm).

ii. **Acceptance criteria:** The sequence of zones present in the chromatogram of the sample solution must exhibit similar pattern to the reference chromatogram. Other faint bands may be present in the chromatogram of the sample solution. See *Annex* for the reference chromatogram.

FOREIGN MATTER

<2> not more than 2%

LOSS ON DRYING/MOISTURE CONTENT

<3> not more than 10%

ALCOHOL (95% v/v)-SOLUBLE EXTRACTIVES

<4> not less than 7%

WATER-SOLUBLE EXTRACTIVES

<4> not less than 10%

TOTAL ASH

<5> not more than 13%

ACID-INSOLUBLE ASH

<5> not more than 6%

WATER-SOLUBLE ASH

<5> not more than 9%

TEST FOR TOXIC HEAVY METALS

<54> The toxic heavy metal limits should conform to the specified limits stated in AO 184 s. 2004. Sample should not have more than 0.3 ug/g (ppm) for arsenic and cadmium, 10 ug/g (ppm) for lead, and 0.5 ug/g (ppm) for mercury.

AFLATOXIN

<57> Total aflatoxin shall conform to the prescribed specification of 20 mcg/kg and aflatoxin B1 of 10 mcg/kg by ELISA method or liquid chromatography

PESTICIDE LIMITS

<56> shall conform to the limits defined by WHO/FAO Codex Alimentarius

MICROBIAL LIMIT

<52> The total aerobic microbial count does not exceed 10^7 cfu/g, the total combined molds and yeast count does not exceed 10^4 cfu/g, and it meets the requirements of the tests for the absence of *Salmonella* species and *Escherichia coli.* Other enterobacteria do not exceed 10^4 cfu/g.

TRADITIONAL USES

Traditionally used as a spice and coloring agent. It is used to treat chronic inflammatory diseases (e.g. chronic obstructive airway disease, asthma), and rheumatoid arthritis, chronic anterior uveitis, conjunctivitis, chicken pox, wound healing, UTI, and gall bladder and liver ailments. Also used for ulcerative colitis and irritable bowel syndrome.

LEUCAENAE LEUCOCEPHALAE

Leucaenae Leucocephalae Semen

Ipil-Ipil Seeds

DEFINITION

Leucaenae Leucocephalae Semen is the dried mature seed of *Leucaena leucocephala* (Lam.) de Witt (Fam. Leguminosae/Fabaceae).

SCIENTIFIC NAME

Leucaena leucocephala (Lam.) de Witt

SYNONYMS

Acacia leucocephala (Lam.) Link
Leucaena glabra Benth.
Mimosa leucocephala Lam.

SELECTED COMMON NAMES

Filipino: *agoho* (P. Bis.), *aghog* (P. Bis.), *Ipel* (Tag.), *kabahero* (C. Bis.), *kariskis* (Ilk.), *kompokompitis* (Ilk.), *loyloy* (S. L. Bis.), *San Pedro* (P. Bis.); *jumpy bean*, *lead tree*, *leucaena*, *wild tamarind* (Eng.), *Santa Elena* (Spa.)

MAJOR CHEMICAL CONSTITUENTS

Leucaenae Leucocephalae Semen is rich in protein with mimosine being the most abundant free amino acid making up 60% of the total free amino acid present in the seeds. Oil obtained from the seeds has significant amounts of linoleic, arachidic, and lignoceric acids. β-sitosterol, α-tocopherol, oxalic acids, and other vitamins such as thiamine, riboflavin, niacin, beta carotenes, and ascorbic acid are also found in the extracts of Leucaenae Leucocephalae Semen.

MORPHOLOGICAL DESCRIPTION OF THE PLANT

Leucaena leucocephala is a fast growing shrub or a small tree, ranging from 2-6 m high. Leaves are bipinnately compound, measuring 3-5 cm long, with leaflets 20 to 30, linear to oblong, or sharply pointed, with sides straight not incurved), unequally sided, measuring 7-12 mm long. Rachis of leaflets are hairy. The long peduncled cluster of flowers appears as a solitary globose head inflorescence, borne axillarily bearing many white colored florets. Stamens are free, inserted and ovary is with a stalk. Sepals and petals are both free (sepal and petal parts not united). Fruit is a pod.

MACROSCOPIC DESCRIPTION

Fruit is a thin, flat, thin, straped shaped , acuminate pod, containing 15-25 elliptic, compressed shining chocolate brown seeds.

MICROSCOPIC DESCRIPTION

The seed of *Leucaena leucocephala* has a unique seed coat having an outer epidermis appearing as a layer of hourglass cells while the inner epidermis has vascular bundles with xylem at the center being surrounded by phloem. Large parenchyma cells dominate the endosperm and cotyledons.

WARNING

May trigger stomachache and diarrhea. Large quantities are toxic to livestock. Teratogenic effects demonstrated in pigs and rats.

PACKAGING AND STORAGE

Preserve in well-closed, light-resistant containers. Protect from moisture.

LABELING

Print the official title and the national title of the herbal material on the label as directed under *General Notices and Requirements* Part I.

TRADITIONAL USES

It is used as treatment for stomachache, ascariasis, and trichinosis. It has been used as antimicrobial, antihelminthic, anti-proliferative, antidiabetic, anti-inflammatory, antioxidant, antitumor, nematicide, and hepatoprotective.

Leucaenae Leucocephalae Semen Pulveratum
Ipil-Ipil Seeds Powder

DEFINITION

Leucaenae Leucocephalae Semen reduced to powder form.

SCIENTIFIC NAME

Leucaena leucocephala (Lam.) de Witt

SYNONYMS

Acacia leucocephala (Lam.) Link
Leucaena glabra Benth.
Mimosa leucocephala Lam.

SELECTED COMMON NAMES

Filipino: *agoho* (P. Bis.), *aghog* (P. Bis.), *Ipel* (Tag.), *kabahero* (C. Bis.), *kariskis* (Ilk.), *kompokompitis* (Ilk.), *loyloy* (S. L. Bis.), *San Pedro* (P. Bis.); *jumpy bean*, *lead tree*, *leucaena*, *wild tamarind* (Eng.), *Santa Elena* (Spa.)

MAJOR CHEMICAL CONSTITUENTS

Leucaenae Leucocephalae Semen is rich in protein with mimosine being the most abundant free amino acid making up 60% of the total free amino acid present in the seeds. Oil obtained from the seeds has significant amounts of linoleic, arachidic, and lignoceric acids. β-sitosterol, α-tocopherol, oxalic acids, and other vitamins such as thiamine, riboflavin, niacin, beta carotenes, and ascorbic acid are also found in the extracts of Leucaenae Leucocephalae Semen

WARNING

May trigger stomachache and diarrhea. Large quantities are toxic to livestock. Teratogenic effects demonstrated in pigs and rats.

PACKAGING AND STORAGE

Preserve in well-closed, light-resistant containers.

LABELING

Print the official title and the national title of the herbal material on the label as directed under *General Notices and Requirements* Part I.

IDENTIFICATION

a. Standard Solution

1 mg/mL of β-sitosterol in methanol. Sonicate until the solute is completely dissolved.

b. Sample Solution

Sonicate 2.5 g of *Leucaena leucocephala* seeds, finely powdered, in 25 mL methanol for 30 min and mix by vortex every 15 min. Filter the mixture using filter paper and use the collected filtrate.

c. Chromatographic System

i. **Adsorbent:** TLC plate silica gel 60 F254 (20x10 cm)

ii. **Application volume:** 0.2 µL sprayed as 9 mm bands using suitable device

iii. **Relative humidity:** Condition the plate to a relative humidity of about 33% for 20 min using a suitable device.

iv. **Developing solvent system:** a mixture of hexane, ethyl acetate, formic acid (41.80:22.86:2.44).

v. **Developing distance:** 85 mm

vi. **Derivatization reagent:** Anisaldehyde-sulfuric acid reagent – Mix in order of 85 mL cold methanol, 10 mL glacial acetic acid, 0.5 mL p-anisaldehyde, and 5 mL concentrated sulfuric acid (perform in ice bath).

d. Analysis

i. **Samples:** *Standard Solution and Sample Solution* Apply the samples to the TLC plate and pre-dry. Develop the chromatogram in a chamber saturated for 30 min using a suitable device and remove the developed plate after drying. Examine under normal light, short wavelength UV light (254 nm), and long wavelength UV light (366 nm). Treat with derivatization reagent, and dry at 100°C for 10 mins. Examine again under normal light, short wavelength UV light (254 nm), and long wavelength UV light (366 nm).

ii. **Acceptance criteria:** The sequence of zones present in the chromatogram of the sample solution must exhibit similar pattern to the reference chromatogram. Other faint bands may be present in the chromatogram of the sample solution. See *Annex* for the reference chromatogram.

FOREIGN MATTER

<2> not more than 1%

LOSS ON DRYING/MOISTURE CONTENT

<3> not more than 10%

ALCOHOL (95% v/v)-SOLUBLE EXTRACTIVES

<4> not less than 5%

WATER-SOLUBLE EXTRACTIVES

<4> not less than 18%

TOTAL ASH

<5> not more than 6%

ACID-INSOLUBLE ASH

<5> not more than 2%

WATER-SOLUBLE ASH

<5> not more than 2%

TEST FOR TOXIC HEAVY METALS

<54> The toxic heavy metal limits should conform to the specified limits stated in AO 184 s. 2004. Sample should not have more than 0.3 ug/g (ppm) for arsenic and cadmium, 10 ug/g (ppm) for lead, and 0.5 ug/g (ppm) for mercury.

AFLATOXIN

<57> Total aflatoxin shall conform to the prescribed specification of 20 mcg/kg and aflatoxin B1 of 10 mcg/kg by ELISA method or liquid chromatography

PESTICIDE LIMITS

<56> shall conform to the limits defined by WHO/FAO Codex Alimentarius

MICROBIAL LIMIT

<52> The total aerobic microbial count does not exceed 10^7 cfu/g, the total combined molds and yeast count does not exceed 10^4 cfu/g, and it meets the requirements of the tests for the absence of *Salmonella* species and *Escherichia coli.* Other enterobacteria do not exceed 10^4 cfu/g.

TRADITIONAL USES

It is used as treatment for stomachache, ascariasis, and trichinosis. It has been used as antimicrobial, antihelminthic, anti-proliferative, antidiabetic, anti-inflammatory, antioxidant, antitumor, nematicide, and hepatoprotective.

MORINGAE OLEIFERAE

Moringae Oleiferae Folium
Malunggay Leaf

DEFINITION

Moringae Oleiferae Folium is the dried leaf of *Moringa oleifera* Lam. (Fam. Moringaceae).

SCIENTIFIC NAME

Moringa oleifera Lam.

SYNONYMS

Guilandina moringa L.
Hyperanthera moringa (L.) Vahl
Moringa zeylanica Burmann

SELECTED COMMON NAMES

Filipino: *arungain* (Pang.), *balungai*, *kamalongan* (P. Bis.), *dool*, *malungit* (Pamp., Bis.), *kalamungai* (C. Bis.), *kalungay* (Bik., Bis., Tag.) *kamalungay* (Ak., Cap., Mas., Sor., Pamp., Tag.), *kamungay* (Dav., Lay., Sam., Sul., Taw., Zambo), kilot (Sul., Taw.), *malongai*, *malungay* (Tag.), *malungue* (Pamp.), *marongoi* (Sbl.), *marunggay* (Ilk., Ibn.), *horse-radish tree* (Eng.), *Horseradish tree*, *drumstick tree*, *West Indian Ben* (En). *Ben ailé* (Fr). Indonesia: *kelor*, *marunga* (Timor). Malaysia: *meringgai*,

gemunggai. Myanmar: *dandalonbin*. Cambodia: *mrum'*. Laos: *'ii h'um*. Thailand: *marum* (general), *phakihum* (north-eastern), *makhonkom* (northern). Vietnam: *ch[uf] m ng[aas]y*.

MAJOR CHEMICAL CONSTITUENTS

Moringa oleifera leaves contain glucosinolates, flavonoids and phenolic acids such as myrecytin, quercetin, kaempferol, ascorbic acid, chlorogenic acid, caffeic acid, and gallic acid with the latter being the most abundant phenolic acid present in dried leaves. These polyphenols are responsible for the antioxidant activity of *Moringa oleifera* leaves. Glucosinolates is found on almost every part of *Moringa oleifera* and 4-O-(a-L-rhamnopyranosyloxy)-benzylglucosinolate (glucomoringin) is the most predominant form in the plant. Isothiocyanates, nitriles, and thiocarbamates that are responsible for the hypotensive properties of Moringa oleifera leaves are produced through the enzymatic catabolism of glucosinolates. The leaves also exhibited cholesterol lowering effects in some studies which is attributed to presence of β-sitosterol.

MORPHOLOGICAL DESCRIPTION OF THE PLANT

Moringa oleifera tree/ shrub eaches a height of 9 m and has a corky gray bark and soft wood. Sweet scented, pea-liked creamy white flowers are supported by a five lobed linear lanceolate calyx (sepals). Inflorescence is a panicle where florets show five unequal, narrowly spatulated petals and hold 5 fertile stamens alternating with staminodes on one side. It has triangular, one-celled, dagger-liked, pendulous pods that dehisce violently, releasing numerous oily black seeds in three angled winged seed coats.

MACROSCOPIC DESCRIPTION

The horseradish smelling alternate leaves are bi-pinnately or tri-pinnately compound, rachis ranging from 12-25 cm long, pubescent, and have three to nine pale green oval/obovate shaped leaflets as the final pinnules. Each leaflet has a swollen bulge (pulvinus) at its base.

MICROSCOPIC DESCRIPTION

The *malunggay* leaf has large single-layered epidermal cells (uniseriate) in both the upper and lower epidermis. Beneath the upper epidermis are highly dense closely packed chloroplasts filling up the palisade layer. Chloroplasts are also prominent in the spongy cells. Stomata are found in the lower epidermis (hypostomatic). Collateral vascular bundles (Xylem region near the palisade layer while phloem near the spongy layer) have been observed

WARNING

The long-term safety of its herbal preparation is unknown.

PACKAGING AND STORAGE

Preserve in well-closed, light-resistant containers.

LABELING

Print the official title and the national title of the herbal material on the label as directed under *General Notices and Requirements* Part I.

TRADITIONAL USES

It is used for diabetes, hyperlipidemia, wound healing, disinfectant, fever, constipation, muscle pains, burns, labor pain, hypertension, malaria, stomach disorder, asthma, and skin problems.

Moringae Oleiferae Folium Pulveratum
Malunggay Leaf Powder

DEFINITION

Moringae Oleiferae Folium reduced to powder form.

SCIENTIFIC NAME

Moringa oleifera Lam.

SYNONYMS

Guilandina moringa L.
Hyperanthera moringa (L.) Vahl
Moringa zeylanica Burmann

SELECTED COMMON NAMES

Filipino: *arungain* (Pang.), *balungai*, *kamalongan* (P. Bis.), *dool*, *malungit* (Pamp., Bis.), *kalamungai* (C. Bis.), *kalungay* (Bik., Bis., Tag.) *kamalungay* (Ak., Cap., Mas., Sor., Pamp., Tag.), *kamungay* (Dav., Lay., Sam., Sul., Taw.,

Zambo), kilot (Sul., Taw.), *malongai*, *malungay* (Tag.), *malungue* (Pamp.), *marongoi* (Sbl.), *marunggay* (Ilk., Ibn.), *horse-radish tree* (Eng.), *Horseradish tree*, *drumstick tree*, *West Indian Ben* (En). *Ben ailé* (Fr). Indonesia: *kelor*, *marunga* (Timor). Malaysia: *meringgai*, *gemunggai*. Myanmar: *dandalonbin*. Cambodia: *mrum'*. Laos: *'ii h'um*. Thailand: *marum* (general), *phakihum* (north-eastern), *makhonkom* (northern). Vietnam: *ch[uf] m ng[aas]y*.

MAJOR CHEMICAL CONSTITUENTS

Moringae Oleifera Folium contain glucosinolates, flavonoids and phenolic acids such as myrecytin, quercetin, kaempferol, ascorbic acid, chlorogenic acid, caffeic acid, and gallic acid with the latter being the most abundant phenolic acid present in dried leaves. These polyphenols are responsible for the antioxidant activity of Moringae Oleifera Folium. Glucosinolates is found on almost every part of Moringa Oleiferae and 4-O-(a-L-rhamnopyranosyloxy)-benzylglucosinolate (glucomoringin) is the most predominant form in the plant. Isothiocyanates, nitriles, and thiocarbamates that are responsible for the hypotensive properties of Moringae Oleiferae Folium are produced through the enzymatic catabolism of glucosinolates. The leaves also exhibited cholesterol lowering effects in some studies which is attributed to presence of β-sitosterol.

WARNING

The long-term safety of its herbal preparation is unknown.

PACKAGING AND STORAGE

Preserve in well-closed, light-resistant containers.

LABELING

Print the official title and the national title of the herbal material on the label as directed under *General Notices and Requirements* Part I.

IDENTIFICATION

a. Standard Solution

1 mg/mL of B-sitosterol in methanol. Sonicate until the solute is completely dissolved.

b. Sample Solution

Sonicate 2.5 g of *Moringa oleifera* Leaf, finely powdered, in 25 mL methanol for 30 min and mix by vortex every 15 min. Filter the mixture using filter paper and use the collected filtrate.

c. Chromatographic System

i. **Adsorbent:** TLC plate silica gel 60 F254 (20x10 cm)

ii. **Application volume:** 0.2 µL sprayed as 9 mm bands using suitable device.

iii. **Relative humidity:** Condition the plate to a relative humidity of about 33% for 20 min using a suitable device.

iv. **Developing solvent system:** a mixture of hexane, ethyl acetate, formic acid (69.48:29.78:0.74).

v. **Developing distance:** 85 mm

vi. **Derivatization reagent:** Anisaldehyde-sulfuric acid reagent – Mix in order of 85 mL cold methanol, 10 mL glacial acetic acid, 0.5 mL p-anisaldehyde, and 5 mL concentrated sulfuric acid (perform in ice bath).

d. Analysis

i. **Samples:** *Standard Solution and Sample Solution* Apply the samples to the TLC plate and pre-dry. Develop the chromatogram in a chamber saturated for 30 min using a suitable device and remove the developed plate after drying. Examine under normal light, short wavelength UV light (254 nm), and long wavelength UV light (366 nm). Treat with derivatization reagent, and dry at 100°C for 10 mins. Examine again under normal light, short wavelength UV light (254 nm), and long wavelength UV light (366 nm).

ii. **Acceptance criteria:** The sequence of zones present in the chromatogram of the sample solution must exhibit similar pattern to the reference chromatogram. Other faint bands may be present in the chromatogram of the sample solution. See *Annex* for the reference chromatogram.

FOREIGN MATTER

<2> not more than 2%

LOSS ON DRYING/MOISTURE CONTENT

<3> not more than 10%

ALCOHOL (95% v/v)-SOLUBLE EXTRACTIVES

<4> not less than 5%

WATER-SOLUBLE EXTRACTIVES

<4> not less than 22%

TOTAL ASH

<5> not more than 14%

ACID-INSOLUBLE ASH

<5> not more than 5%

WATER-SOLUBLE ASH

<5> not more than 7%

TEST FOR TOXIC HEAVY METALS

<54> The toxic heavy metal limits should conform to the specified limits stated in AO 184 s. 2004. Sample should not have more than 0.3 ug/g (ppm) for arsenic and cadmium, 10 ug/g (ppm) for lead, and 0.5 ug/g (ppm) for mercury.

AFLATOXIN

<57> Total aflatoxin shall conform to the prescribed specification of 20 mcg/kg and aflatoxin B1 of 10 mcg/kg by ELISA method or liquid chromatography

PESTICIDE LIMITS

<56> shall conform to the limits defined by WHO/FAO Codex Alimentarius

MICROBIAL LIMIT

<52> The total aerobic microbial count does not exceed 10^7 cfu/g, the total combined molds and yeast count does not exceed 10^4 cfu/g, and it meets the requirements of the tests for the absence of *Salmonella* species and *Escherichia coli.* Other enterobacteria do not exceed 10^4 cfu/g.

TRADITIONAL USES

It is used for diabetes, hyperlipidemia, wound healing, disinfectant, fever, constipation, muscle pains, burns, labor pain, hypertension, malaria, stomach disorder, asthma, and skin problems.

PSIDII GUAJAVAE

Psidii Guajavae Folium
Bayabas Leaf

DEFINITION

Psidii Guajavae Folium is the dried leaf of *Psidium guajava* L. (Fam. Myrtaceae).

SCIENTIFIC NAME

Psidium guajava L.

SYNONYMS

Psidium angustifolium Lam.
Psidium cujavillus Burm.f.
Guajava pumila (Vahl) Kuntze
Guajava pyrifera (L.) Kuntze
Myrtus guajava (L.) Kuntze

SELECTED COMMON NAMES

Filipino: *aykud* (If. Kal.), *bagabas* (Ig.), *bayabo* (Ibn.), *bayauas* (Bik. Pang.), *bayawas* (Ak., Ilk.), *biabas* (Sul.), *cayab* (Bon.), *gaiyabat*, *gaiyabit* (If.), *geyabas* (Bon.), *guayabas*, *bayabas*, *kalimbahin*, *tayabas* (Tag.), *guyabas* (Ilk.), *mayabas* (Rom.), *guava* (Eng.), *araza*, *guayabo*, *guayaba* (Spa.), *fan shi liu* (Chi.)

MAJOR CHEMICAL CONSTITUENTS

C-glucosidic ellagitannins are common in the family Myrtaceae to which *Psidium guajava* L. belongs. Casuarinin, guavins A, B, C, and D, pedunculagin, stachyurin, strictinin, isotrictinin, and tellimagrandin have been reported. The leaves yield a volatile oil containing monoterpenes, such as camphene, 1,8-cineole, p-cymene, menthol, α- and β-pinene; sesquiterpenes such as armadendrene, β-bisabolene, δ-cadinene, caryophyllene, α- and β-copaene, and δ-elemene. Other compounds that have been identified in the leaves are flavonoid glycosides (and their aglycones), such as myricitrin (myricetin), quercitrin and isoquercitrin (quercetin); amritroside, coumarin; triterpenes and phytosterols such as α- and β-amyrin with their corresponding acetates, and ursolic acid. Two new tripenoids. guajavolide and guajavenoic acid, together with oleanolic acid, have recently been isolated from the fresh leaves.

MORPHOLOGICAL DESCRIPTION OF THE PLANT

Psidium guajava is an introduced Tropical American shrub or small tree that reaches a height of 8-10 m. Its bark has a peculiar greenish to red brownish color and somewhat with smooth texture. The branches, usually four-angled especially when young, give rise to leaves that are borne in two rows per node (where two leaves are attached to the stem in an opposite manner). One solitary or two to three white flowers are borne by a peduncle, with in-curved petals, each of which is surrounded by numerous stamens. Ovary is inferior, developing into a round or obovoid green fleshy berry fruit, 4-5 cm long that turns yellow upon maturity. Typical of a berry type of fruit, seeds are numerous.

MACROSCOPIC DESCRIPTION

Leaves are usually 5 -10 cm long, attached to the stem in an opposite manner, with oblong to elliptic shape, where leaf tip is pointed (acuminate) while its base is rounded.

MICROSCOPIC DESCRIPTION

The leaf of *Psidium guajava* has a multiseriate type of epidermis in its upper layer, with two to three layers of cells containing tannins and oils within the secretory cells present. The mesophyll has the spongy layer in between two layers of palisade cells (i.e. isobilateral type of mesophyll). The midrib has bicollateral type of vascular bundles surrounded by a sclerenchymatous bundle sheath (or pericyclic fibers). The midrib has a single layer of cells in the lower epidermis (i.e. uniseriate) and unicellular unbranched trichomes. Stomata can be found in both lower and upper epidermis of the leaf (amphistomatic), where the subsidiary cells surrounding the stomatal pore are not different from other neighboring epidermal cells (anomocytic stomata). Clusters of large calcium oxalate containing rosette crystals are also present.

WARNING

Excessive *bayabas* leaves were found to cause constipation, ulcer, nausea, and vomiting.

PACKAGING AND STORAGE

Preserve in well-closed, light-resistant containers.

LABELING

Print the official title and the national title of the herbal material on the label as directed under *General Notices and Requirements* Part I.

TRADITIONAL USES

Used for treating gastrointestinal infections, malaria, and respiratory, oral and dermal infections. It has antidiabetic, antihypertensive, and anticancer properties.

Psidii Guajavae Folium Pulveratum

Bayabas Leaf Powder

DEFINITION

Psidii Guajavae Folium reduced to powder form.

SCIENTIFIC NAME

Psidium guajava L.

SYNONYMS

Psidium angustifolium Lam.
Psidium cujavillus Burm.f.
Guajava pumila (Vahl) Kuntze
Guajava pyrifera (L.) Kuntze
Myrtus guajava (L.) Kuntze

SELECTED COMMON NAMES

Filipino: *aykud* (If. Kal.), *bagabas* (Ig.), *bayabo* (Ibn.), *bayauas* (Bik. Pang.), *bayawas* (Ak., Ilk.), *biabas* (Sul.), *cayab* (Bon.), *gaiyabat*, *gaiyabit* (If.), *geyabas* (Bon.), *guayabas*, *bayabas*, *kalimbahin*, *tayabas* (Tag.), *guyabas* (Ilk.), *mayabas* (Rom.), *guava* (Eng.), *araza*, *guayabo*, *guayaba* (Spa.), *fan shi liu* (Chi.)

MAJOR CHEMICAL CONSTITUENTS

C-glucosidic ellagitannins are common in the family Myrtaceae to which *Psidium guajava* L. belongs. Casuarinin, guavins A, B, C, and D, pedunculagin, stachyurin, strictinin, isotrictinin, and tellimagrandin have been reported. The leaves yield a volatile oil containing monoterpenes, such as camphene, 1,8-cineole, p-cymene, menthol, α- and β-pinene; sesquiterpenes such as armadendrene, β-bisabolene, δ-cadinene, caryophyllene, α- and β-copaene, and δ-elemene. Other compounds that have been identified in the leaves are flavonoid glycosides (and their aglycones), such as myricitrin (myricetin), quercitrin and isoquercitrin (quercetin); amritroside, coumarin; triterpenes and phytosterols such as α- and β-amyrin with their corresponding acetates, and ursolic acid. Two new tripenoids. guajavolide and guajavenoic acid, together with oleanolic acid, have recently been isolated from the fresh leaves.

WARNING

Exposure to *bayabas* leaf extract might cause temporary nausea or stomach pain in some people. It might also trigger skin irritation.

PACKAGING AND STORAGE

Preserve in well-closed, light-resistant containers.

LABELING

Print the official title and the national title of the herbal material on the label as directed under *General Notices and Requirements* Part I.

IDENTIFICATION

a. Standard Solution

1 mg/mL of gallic acid in methanol. Sonicate until the solute is completely dissolved.

b. Sample Solution

Sonicate 2.5 g of *Psidium guajava* Leaf, finely powdered, in 25 mL methanol for 30 min and mix by vortex every 15 min. Filter the mixture using filter paper and use the collected filtrate.

c. Chromatographic System

i. **Adsorbent:** TLC plate silica gel 60 F254 (20x10 cm)

ii. **Application volume:** 0.2 µL sprayed as 9 mm bands using suitable device

iii. **Relative humidity:** Condition the plate to a relative humidity of about 33% for 20 min using a suitable device.

iv. **Developing solvent system:** a mixture of chloroform, acetone, formic acid (10:2:1)

v. **Developing distance:** 85 mm

vi. **Derivatization reagent:** Anisaldehyde-sulfuric acid reagent – Mix in order of 85 mL cold methanol, 10 mL glacial acetic acid, 0.5 mL p-anisaldehyde, and 5 mL concentrated sulfuricacid (perform in ice bath).

d. Analysis

i. **Samples:** Apply the samples to the TLC plate and pre-dry. Develop the chromatogram in a chamber saturated for 30 min using a suitable device and remove the developed plate after drying. Examine under normal light, short wavelength UV light (254 nm), and long wavelength UV light (366 n m). Treat with derivatization reagent, and dry at 100°C for 10 min. Examine again under normal light, short wavelength UV light (254 nm), and long wavelength UV light (366 nm).

ii. **Acceptance criteria:** The sequence of zones present in the chromatogram of the sample solution must exhibit similar pattern to the reference chromatogram. Other faint bands may be present in the chromatogram of the sample solution. See *Annex* for the reference chromatogram.

FOREIGN MATTER

<2> not more than 2%

LOSS ON DRYING/MOISTURE CONTENT

<3> not more than 10%

ALCOHOL (95% v/v)-SOLUBLE EXTRACTIVES

<4> not less than 6%

WATER-SOLUBLE EXTRACTIVES

<4> not less than 5%

TOTAL ASH

<5> not more than 8%

ACID-INSOLUBLE ASH

<5> not more than 2%

WATER-SOLUBLE ASH

<5> not more than 3%

TEST FOR TOXIC HEAVY METALS

<54> The toxic heavy metal limits should conform to the specified limits stated in AO 184 s. 2004. Sample should not have more than 0.3 ug/g (ppm) for arsenic and cadmium, 10 ug/g (ppm) for lead, and 0.5 ug/g (ppm) for mercury.

AFLATOXIN

<57> Total aflatoxin shall conform to the prescribed specification of 20 mcg/kg and aflatoxin B1 of 10 mcg/kg by ELISA method or liquid chromatography

PESTICIDE LIMITS

<56> shall conform to the limits defined by WHO/FAO Codex Alimentarius

MICROBIAL LIMIT

<52> The total aerobic microbial count does not exceed 10^7 cfu/g, the total combined molds and yeast count does not exceed 10^4 cfu/g, and it meets the requirements of the tests for the absence of *Salmonella* species and *Escherichia coli.* Other enterobacteria do not exceed 10^4 cfu/g.

TRADITIONAL USES

Used for treating gastrointestinal infections, malaria, and respiratory, oral and dermal infections. It has antidiabetic, antihypertensive, and anticancer properties.

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Annex I contains the Plant photographs, HPTLC profiles, and microscopic photographs.

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Annex I

HERBAL MEDICINES

BLUMEAE BALSAMIFERAE FOLIUM | *Sambong Leaf*

SCIENTIFIC NAME: Blumea balsamifera (L.) DC.
COMMON NAME: Sambong
FAMILY: Asteraceae
HABITAT: Open fields, grasslands and waste areas at low and medium altitudes
COLLECTION
Place: Region 4A, UP Biotech, Los Baños, Laguna
Collector: Pharmacopoeia Agri. Team
Date:07/04/21
Certified by: Museum of Natural History, UPLB
Date: 01/19/22

Blumeae Balsamiferae Folium
Sambong Leaf
Blumeae Balsamiferae Folium Pulveratum
Powdered Sambong Leaf
Reference HPTLC

Blumea balsamifera **(Linné) De Candolle (Fam. Compositae/Asteraceae)**

Sample specimen solution: The extraction method used is the same as the *Sample Solution* specified in the individual monograph

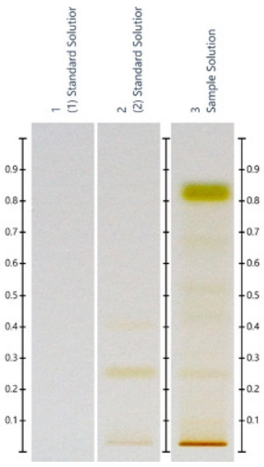
Standard specimen solution: 1.0 mg/mL of Eriodictyol RS in ethanol

Application volume: 0.2 µL sprayed as 9-mm bands

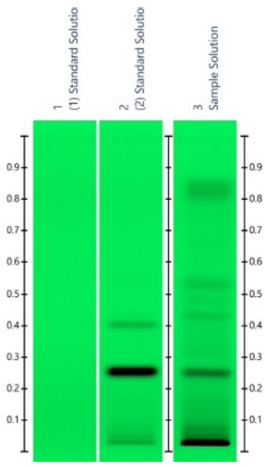
Developing solvent: A mixture of chloroform and methanol (8:1)

Spray reagent: 1% aluminum chloride in methanol

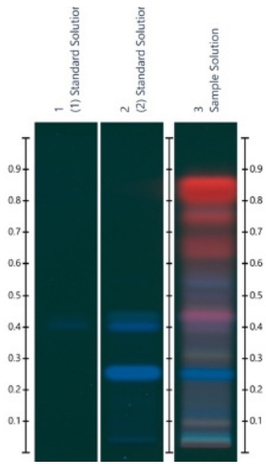
Underivatized



White Light

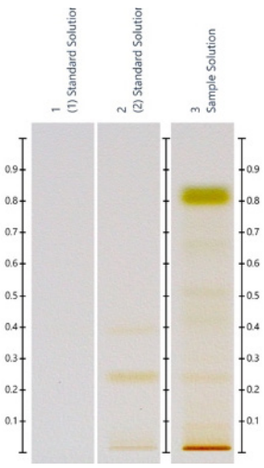


UV-254

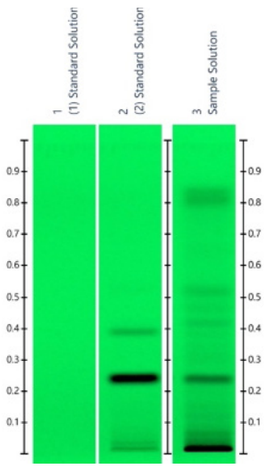


UV-366

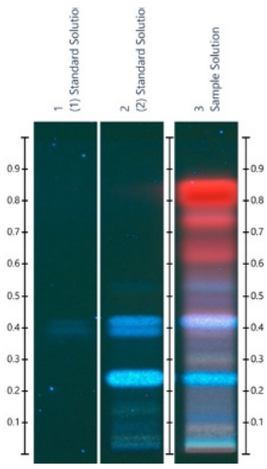
Derivatized



White Light



UV-254



UV-366

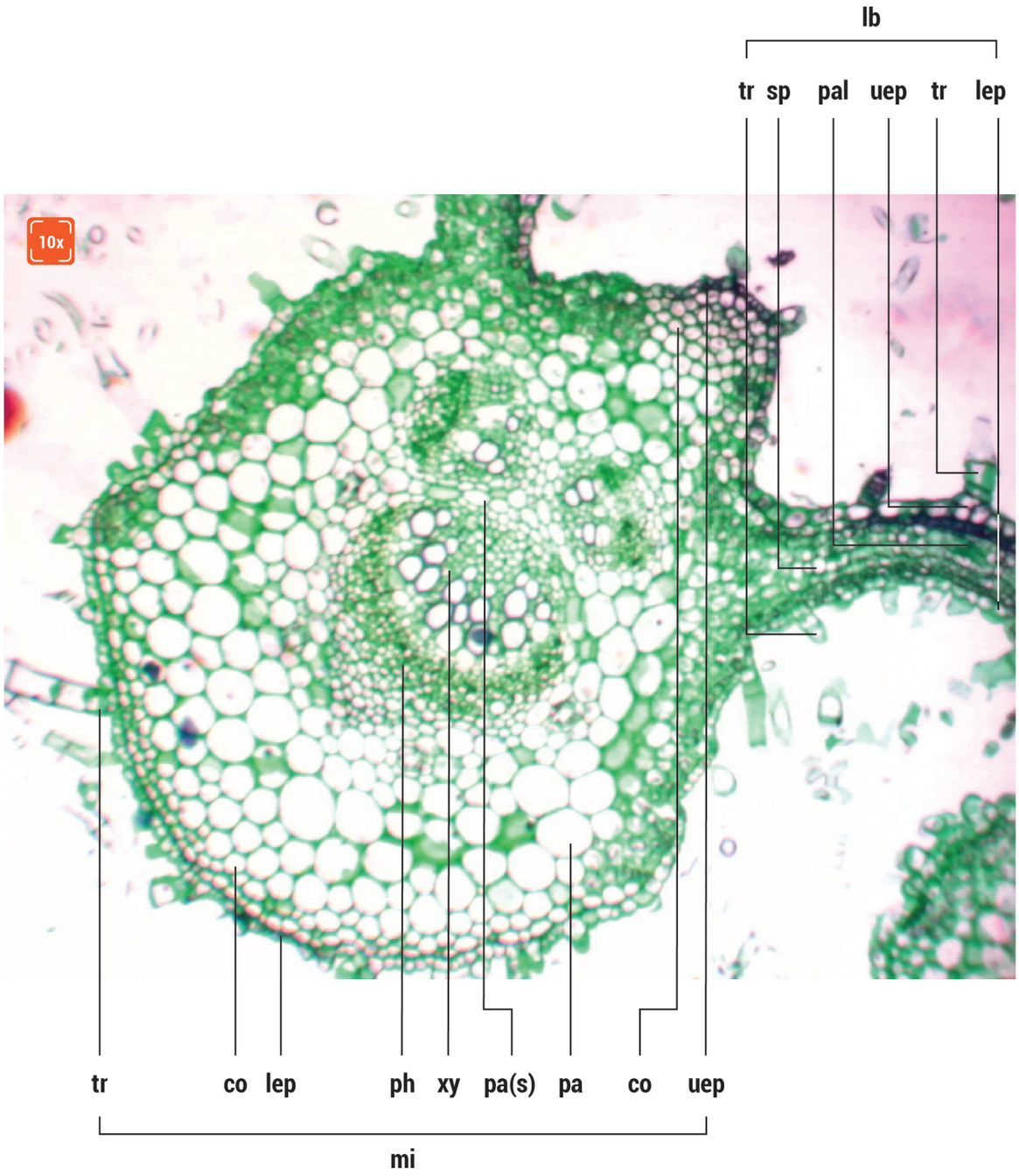
Blumeae Balsamiferae Folium
Sambong Leaf
Blumeae Balsamiferae Folium Pulveratum
Powdered Sambong Leaf
Reference HPTLC

Table 6.1. Retention Factors (R_f) and Colors of Major Bands in Sample Solution

R_f VALUE	UNDERIVATIZED			DERIVATIZED		
	NORMAL LIGHT	UV-254	UV-366	NORMAL LIGHT	UV-254	UV-366
0.85	Green		Red	Green		Red
0.82	Yellow			Yellow		
0.68		Black			Black	
0.66	Yellow			Yellow		
0.62		Black			Black	
0.54	BlueGreen		Red	Blue Green		Red
0.48		Black			Black	
0.46			Blue Green			
0.41			Red			
0.35*		Black			Black	Blue
0.28			Red			Red

*R_f value of Eriodictyol - reference standard

BLUMEAE BALSAMIFERAE FOLIUM | *Sambong Leaf*
MICROSCOPIC EXAMINATION



Cross Section of the Leaf Blade and Midrib

tr: trichome; **uep:** upper epidermis; **pal:** palisade parenchyma; **sp:** spongy parenchyma; **lep:** lower epidermis; **lb:** leaf blade; **co:** collenchyma; **ph:** phloem; **xy:** xylem; **pa(s):** parenchyma (small); **pa:** parenchyma; **mi:** midrib

EHRETIAE MICROPHYLLAE FOLIUM | *Tsaang Gubat Leaf*

SCIENTIFIC NAME: Ehretia microphylla Lam.
COMMON NAME: Tsaang gubat
FAMILY: Boraginaceae
HABITAT: Thickets and secondary forests at low and medium altitudes
COLLECTION:
Place: Region 4A, UP Biotech, Los Baños, Laguna
Collector: Pharmacopoeia Agri. Team
Date: 07/04/21
Certified by: Museum of Natural History, UPLB
Date:

Ehretiae Microphyllae Folium
Tsaang Gubat Leaf
Ehretiae Microphyllae Folium Pulveratum
Powdered *Tsaang Gubat* Leaf
Reference HPTLC

***Ehretia microphylla* Lam. (Fam. Boraginaceae)**

Sample specimen solution: The extraction method used is the same as the *Sample Solution* specified in the individual monograph

Standard specimen solution: (1) 2.0 mg/mL of Caffeic Acid RS in methanol

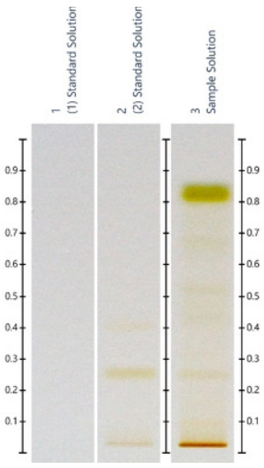
(2) 2.0 mg/mL of Rosmarinic Acid RS in methanoll

Application volume: 0.2 µL sprayed as 9-mm bands

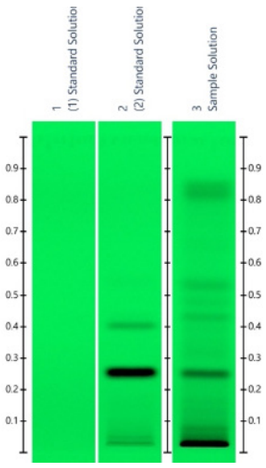
Developing solvent: A mixture of chloroform, ethyl acetate, and formic acid (5:4:1)

Spray reagent: Anisaldehyde-sulfuric acid reagent

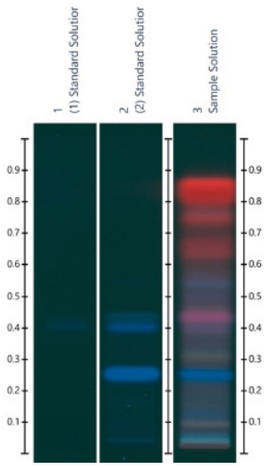
Underivatized



White Light

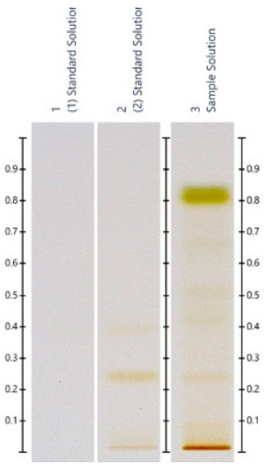


UV-254

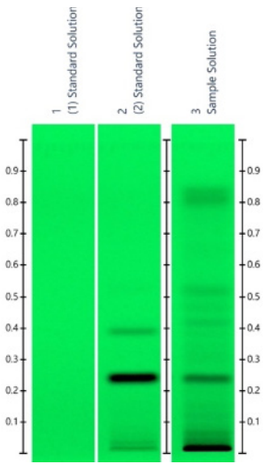


UV-366

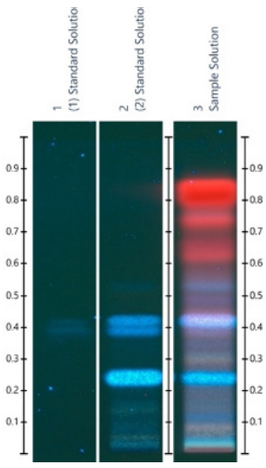
Derivatized



White Light



UV-254



UV-366

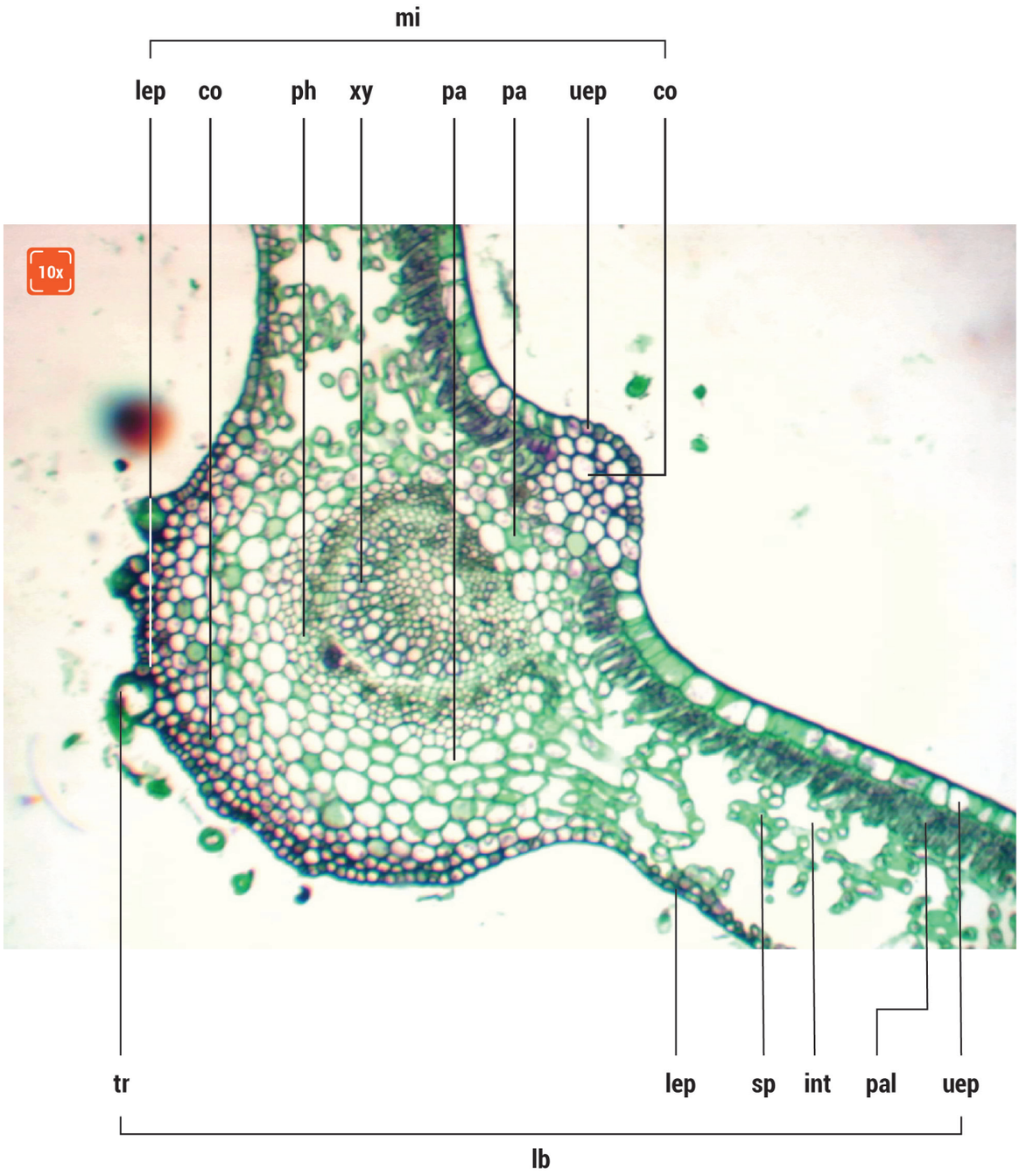
Ehretiae Microphyllae Folium
Tsaang Gubat Leaf
Ehretiae Microphyllae Folium Pulveratum
Powdered *Tsaang Gubat* Leaf
Reference HPTLC

Table 6.2. Retention Factors (R_f) and Colors of Major Bands in Sample Solution

R_f VALUE	UNDERIVATIZED			DERIVATIZED		
	NORMAL LIGHT	UV-254	UV-366	NORMAL LIGHT	UV-254	UV-366
0.80	Yellow	Black	Red	Yellow	Black	Red
0.51		Black	Red		Black	Red
0.43			Pink			Pink
0.42		Black			Black	
0.39*	Dark Yellow	Black	Blue	Dark Yellow	Black	Blue
0.24**	Dark Yellow	Black	Blue	Dark Yellow	Black	Blue

* R_f value of Caffeic acid - reference standard
** R_f value of Rosmarinic acid - reference standard

EHRETIAE MICROPHYLLAE FOLIUM | *Tsaang Gubat Leaf*
MICROSCOPIC EXAMINATION



Cross Section of the Leaf Blade and Midrib

tr: trichome; **uep:** upper epidermis; **pal:** palisade parenchyma; **sp:** spongy parenchyma; **lep:** lower epidermis; **lb:** leaf blade; **co:** collenchyma; **ph:** phloem; **xy:** xylem; **int:** intercellular space; **pa:** parenchyma; **mi:** midrib

MENTHAE ˣVILLOSAE FOLIUM | *Yerba Buena Leaf*

SCIENTIFIC NAME: Mentho x villosa Huds.
COMMON NAME: Yerba buena
FAMILY: Lamiaceae
HABITAT: Cultivated in the Philippines, thrives well at high elevations, rarely flowers in lowlands
COLLECTION:
Place: Region 3, Sta. Rosa, Naeva Ecija (LAC)
Collector: Pharmacopoeia Agri. Team
Date: 06/28/21
Certified by: Museum of Natural History, UPLB
Date:

Menthae [X]Villosae Folium
Yerba Buena Leaf
Menthae [X]Villosae Folium Pulveratum
Powdered Yerba Buena Leaf
Reference HPTLC

Mentha x villosa **Huds. (Fam. Lamiaceae)**

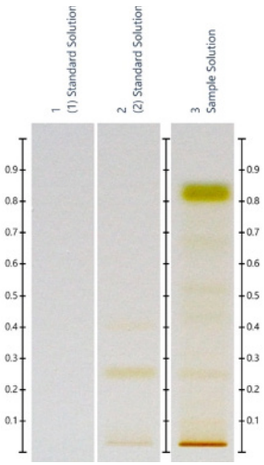
Sample specimen solution: The extraction method used is the same as the *Sample Solution* specified in the individual monograph

Application volume: 0.2 µL sprayed as 9-mm bands

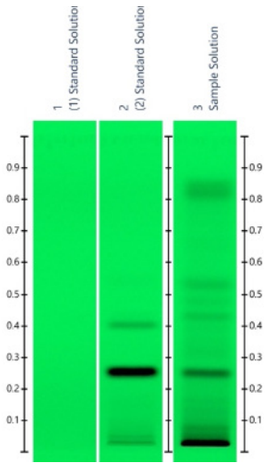
Developing solvent: A mixture of chloroform and ethyl acetate (95:5)

Spray reagent: Anisaldehyde-sulfuric acid reagent

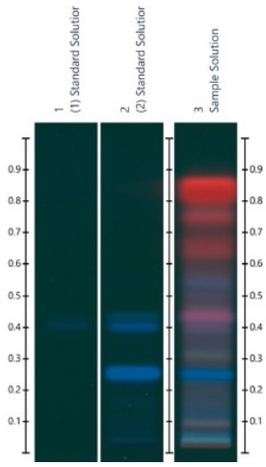
Underivatized



White Light

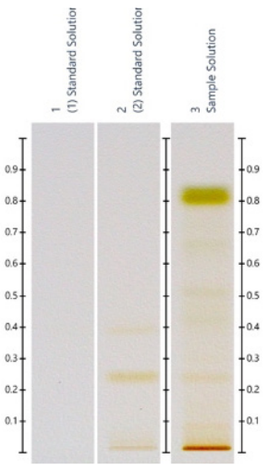


UV-254

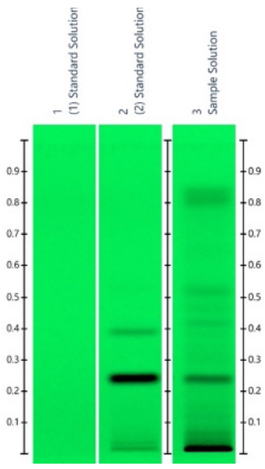


UV-366

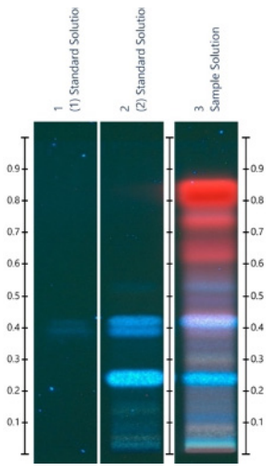
Derivatized



White Light



UV-254



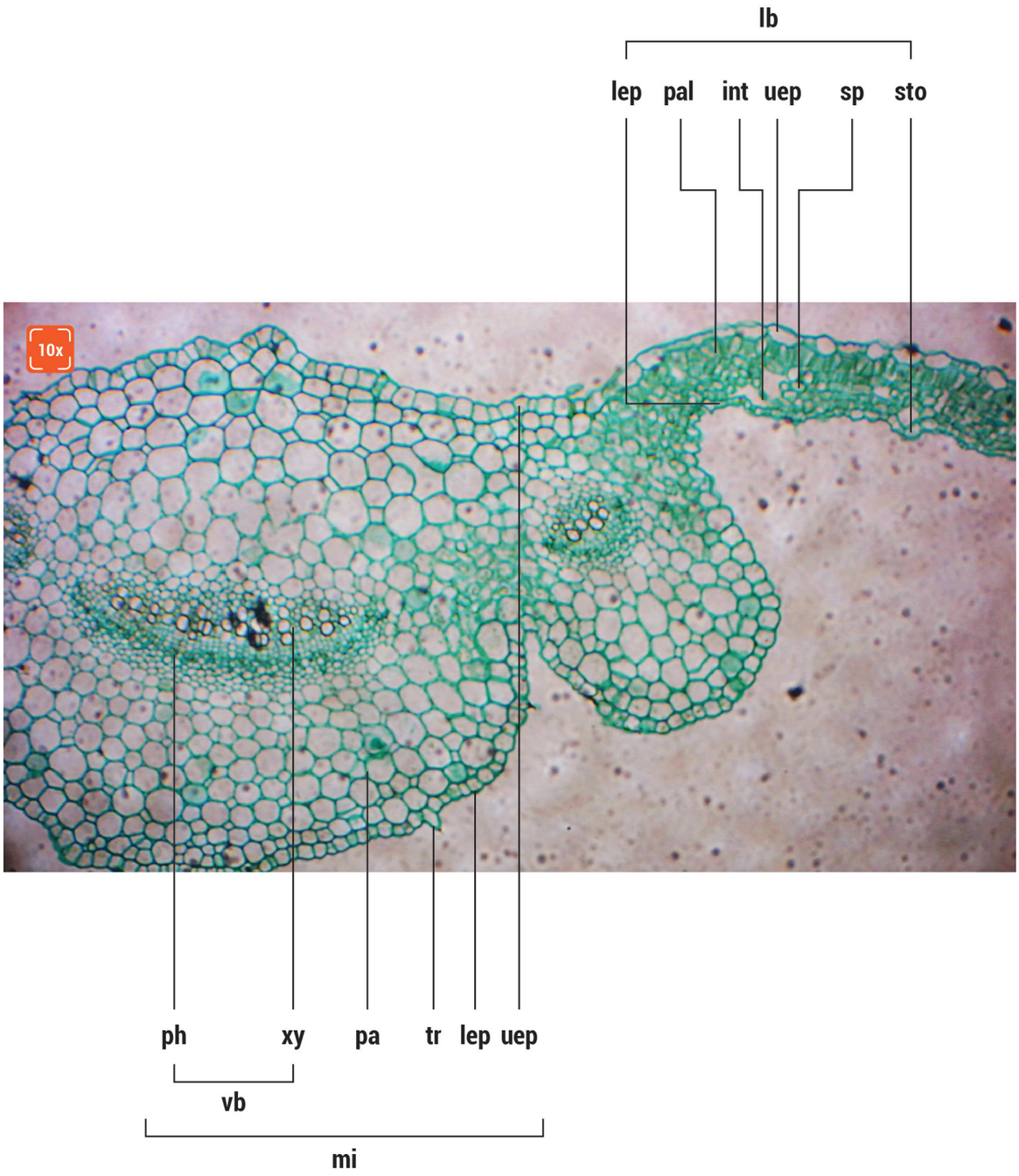
UV-366

Menthae [x]Villosae Folium
Yerba Buena Leaf
Menthae [x]Villosae Folium Pulveratum
Powdered Yerba Buena Leaf
Reference HPTLC

Table 6.3. Retention Factors (R_f) and Colors of Major Bands in Sample Solution

R_f VALUE	UNDERIVATIZED			DERIVATIZED		
	NORMAL LIGHT	UV-254	UV-366	NORMAL LIGHT	UV-254	UV-366
0.81				Red		Pink
0.72		Black				
0.71	Beige			Green		Red
0.66		Black	Red		Blue Green	Blue
0.58		Black	Red			Red
0.56			Blue/Pink			
0.46	Green	Black	Red	Green		
0.38				Brown		White
0.36			Red			
0.34	Yellow	Black		Green		Red
0.21				Brown	Black	White
0.14			Blue			Blue
0.09	Dark Yellow		Red	Green		
0.03			Pink			

MENTHAE ˣVILLOSAE FOLIUM | *Yerba Buena Leaf*
MICROSCOPIC EXAMINATION



Cross Section of the Leaf Blade and Midrib

ph: phloem; **xy:** xylem; **vb:** vascular bundle; **pa:** parenchyma; **tr:** trichome; **lep:** lower epidermis; **uep:** upper epidermis; **mi:** midrib; **pal:** palisade parenchyma; **sp:** spongy parenchyma; **int:** intercelluar space; **sto:** stoma; **lb:** leaf blade

MOMORDICAE CHARANTIAE FOLIUM | *Ampalaya Leaf*

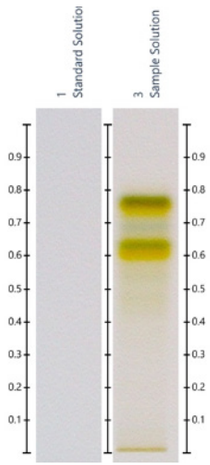
SCIENTIFIC NAME: Momordica charantia L. 'Makiling'
COMMON NAME: Ampalaya
FAMILY: Cucurbitaceae
HABITAT: Cultivated in the Philippines for its leaves, for medicinal purposes
COLLECTION:
Place: Region 5, Amtic, Sabluyon, Ligao, Albay
Collector: Pharmacopoeia Agri. Team
Date: 11/19/21
Certified by: Museum of Natural History, UPLB
Date:

Momordicae Charantiae Folium
Ampalaya Leaf
Momordicae Charantiae Folium Pulveratum
Powdered Ampalaya Leaf
Reference HPTLC

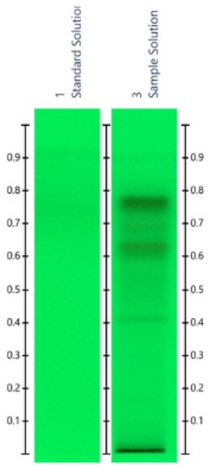
***Momordica charantia* Linné (Fam. Cucurbitaceae)**

Sample specimen solution:	The extraction method used is the same as the *Sample Solution* specified in the individual monograph
Standard specimen solution:	250 mg/L of beta-Sitosterol Glucoside RS in methanol
Application volume:	0.2 µL sprayed as 9-mm bands
Developing solvent:	A mixture of chloroform, ethyl acetate, and formic acid (5:7.5:0.75)
Spray reagent:	Anisaldehyde-sulfuric acid reagent

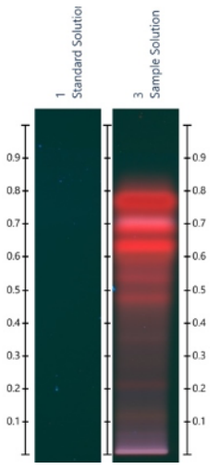
Underivatized



White Light

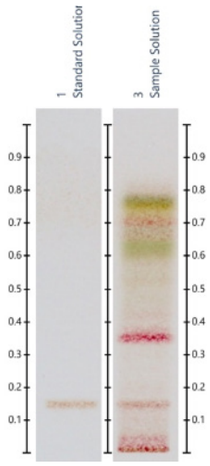


UV-254

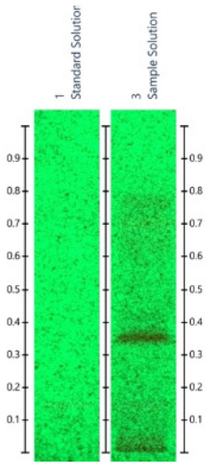


UV-366

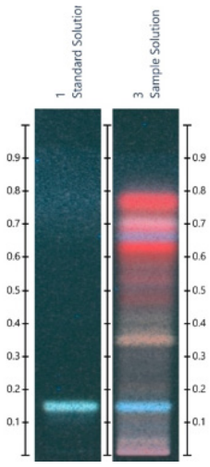
Derivatized



White Light



UV-254



UV-366

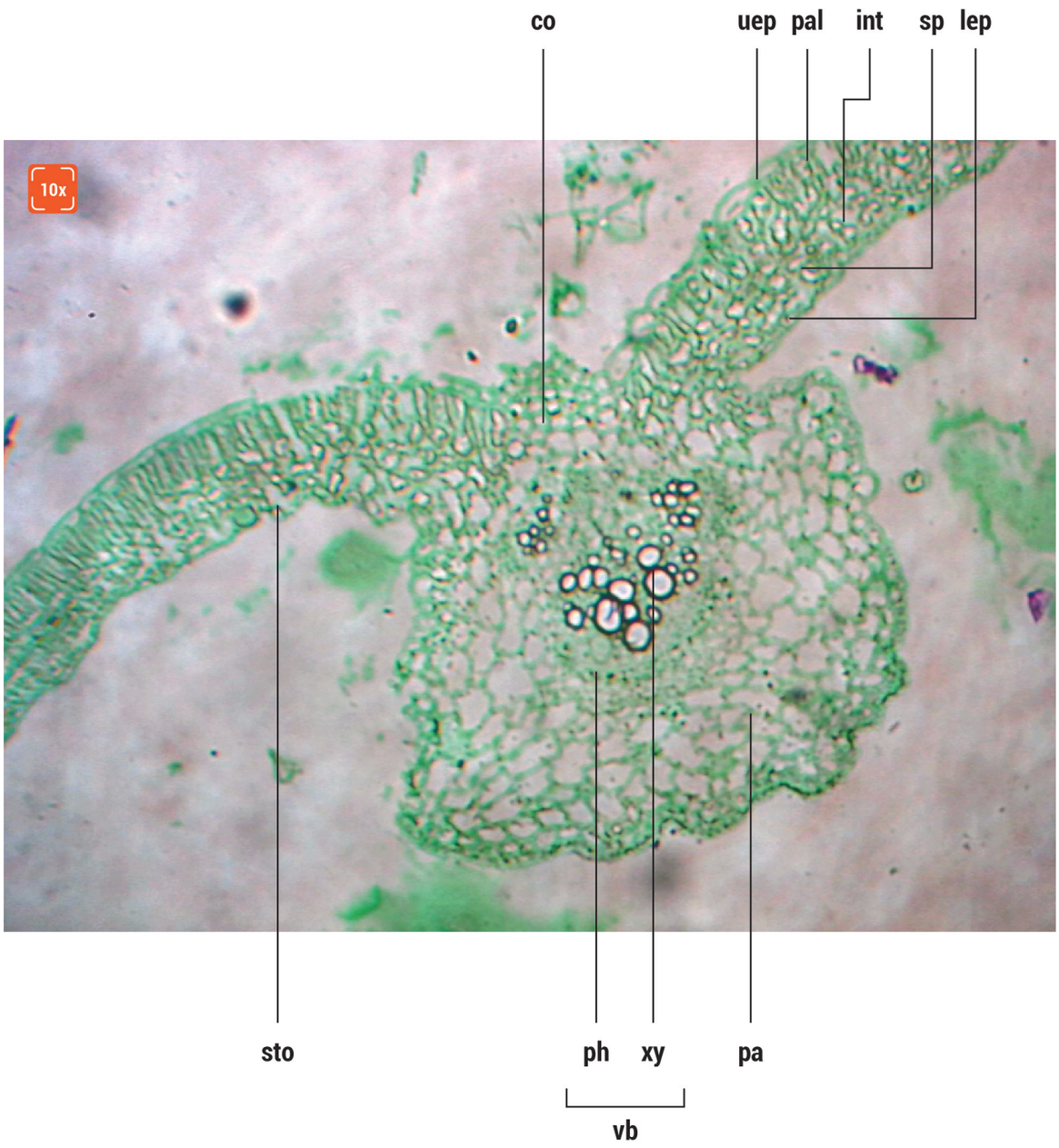
Momordicae Charantiae Folium
Ampalaya Leaf
Momordicae Charantiae Folium Pulveratum
Powdered Ampalaya Leaf
Reference HPTLC

Table 6.4. Retention Factors (R_f) and Colors of Major Bands in Sample Solution

R_f VALUE	UNDERIVATIZED			DERIVATIZED		
	NORMAL LIGHT	UV-254	UV-366	NORMAL LIGHT	UV-254	UV-366
0.75	Green	Black	Red	Green		Red
0.71	Yellow					
0.68			Pink			Pink
0.65						Blue
0.62	Green	Black	Red			Red
0.60				Green		
0.59	Yellow					
0.52			Red			
0.46			Red			
0.40		Black				
0.34				Red	Black	Peach
0.14*				Brown		Light Blue

*R_f value of β-sitosterol glucoside - reference standard

MOMORDICAE CHARANTIAE FOLIUM | *Ampalaya Leaf*
MICROSCOPIC EXAMINATION



Cross Section of the Leaf Blade and Midrib

uep: upper epidermis; **pal:** palisade parenchyma; **int:** intercelluar space; **sp:** spongy parenchyma; **sto:** stoma; **lep:** lower epidermis; **co:** collenchyma; **xy:** xylem; **ph:** phloem; **vb:** vascular bundle; **pa:** parenchyma

PEPEROMIAE PELLUCIDAE HERBA | *Ulasimang Bato Aerial Parts*

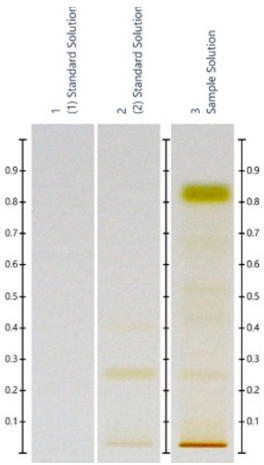
SCIENTIFIC NAME: Peperomia pellucida (L.) Kunth
COMMON NAME: Pansit-pansitan/Ulasimang bato
FAMILY: Piperaceae
HABITAT: Damp, shady and loose soil, rocky parts of canals, nooks of garden and yard
COLLECTION
Place: Region 4A, UP Biotech, Los Baños, Laguna
Collector: Pharmacopoeia Agri. Team
Date: 07/04/21
Certified by: Museum of Natural History, UPLB
Date: 01/19/22

Peperomiae Pellucidae Herba
***Ulasimang Bato* Aerial Parts**
Peperomiae Pellucidae Herba Pulveratum
Powdered *Ulasimang Bato* Aerial Parts
Reference HPTLC

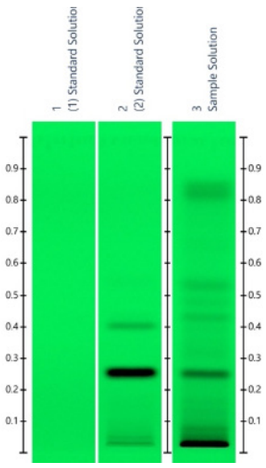
***Peperomia pellucida* (Linné) Humboldt, Bonpland, Kunth (Fam. Piperaceae)**

Sample specimen solution:	The extraction method used is the same as the *Sample Solution* specified in the individual monograph
Standard specimen solution:	1 mg/mL of Dillapiole RS in methanol
Application volume:	0.2 µL sprayed as 9-mm bands
Developing solvent:	A mixture of hexane, ethyl acetate, chloroform, and glacial acetic acid (3:1:1:0.025)
Spray reagent:	Anisaldehyde-sulfuric acid reagent

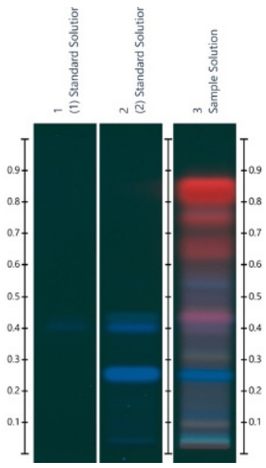
Underivatized



White Light

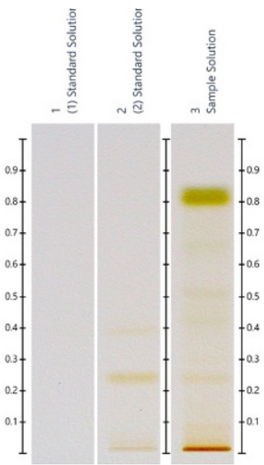


UV-254

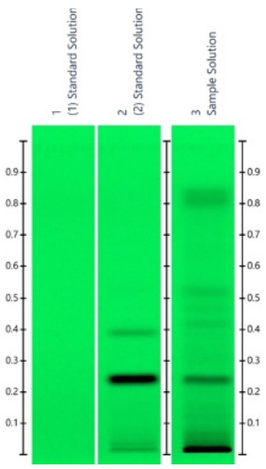


UV-366

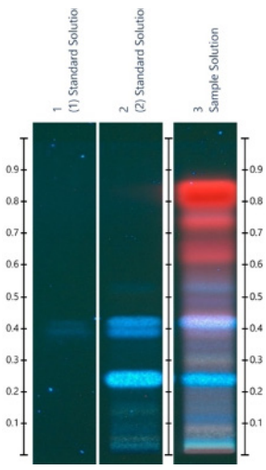
Derivatized



White Light



UV-254



UV-366

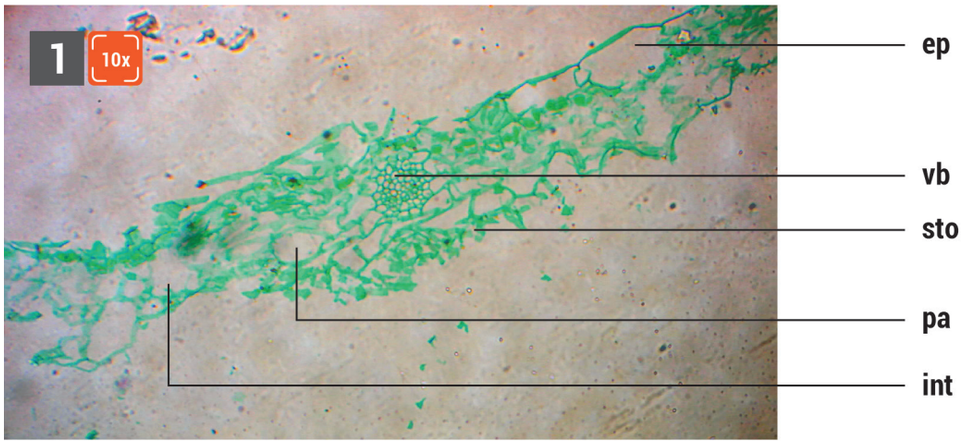
Peperomiae Pellucidae Herba
***Ulasimang Bato* Aerial Parts**
Peperomiae Pellucidae Herba Pulveratum
Powdered *Ulasimang Bato* Aerial Parts
Reference HPTLC

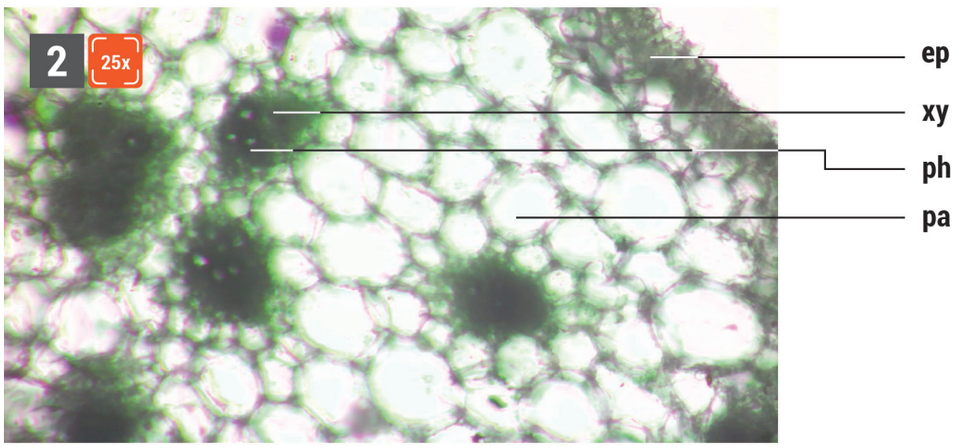
Table 6.5. Retention Factors (R_f) and Colors of Major Bands in Sample Solution

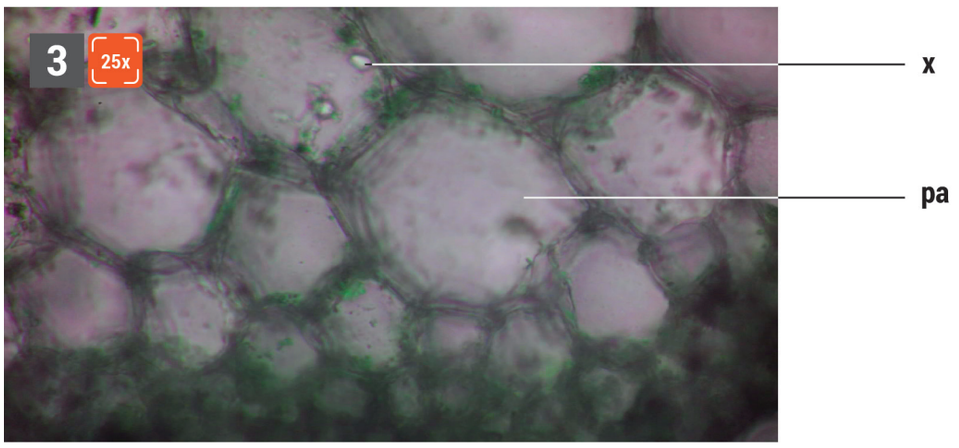
R_f VALUE	UNDERIVATIZED			DERIVATIZED		
	NORMAL LIGHT	UV-254	UV-366	NORMAL LIGHT	UV-254	UV-366
0.73						
0.67			Red			
0.61*		Black		Brown	Black	Dark Blue
0.53		Black				
0.48			Blue			Blue
0.46			Pink/Violet			Pink/Violet
0.45	Brown	Black		Green		
0.33			Red	Brown		Red Orange
0.24	Green	Black	Red	Green		Red
0.20	Green	Black				
0.18			Pink			Pink
0.15	Yellow Green	Black	Red	Yellow Green	Black	Red
0.10	Yellow		White			Light Pink
0.05	Yellow Green					Red

*R_f value of Dillapiole - reference standard

PEPEROMIAE PELLUCIDAE HERBA | *Ulasimang Bato Aerial Parts*
MICROSCOPIC EXAMINATION







1. Cross Section of the Leaf Blade

2. Cross Section of the Stem

3. Cross Section of the Stem with Crystal

ep: epidermis, **vb:** vascular bundle; **sto:** stoma; **pa:** parenchyma; **int:** intercellular space; **xy:** xylem; **ph:** phloem; **x:** crystal

PLECTRANTHI AMBOINICI FOLIUM | *Oregano Leaf*

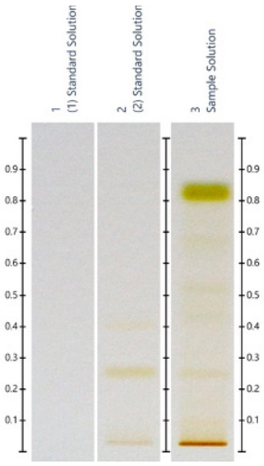
SCIENTIFIC NAME: Plectranthus amboinicus (Lour.) Spreng.
COMMON NAME: Oregano
FAMILY: Lamiaceae
HABITAT: Widely distributed throughout the Philippines, cultivated, introduced
COLLECTION:
Place: Region 4A, Natatas, Tanauan City, Batangas (BINHI)
Collector: Pharmacopoeia Agri. Team
Date: 03/09/21
Certified by: Museum of Natural History, UPLB
Date:

Plectranthi Amboinici Folium
Oregano Leaf
Plectranthi Amboinici Folium Pulveratum
Powdered Oregano Leaf
Reference HPTLC

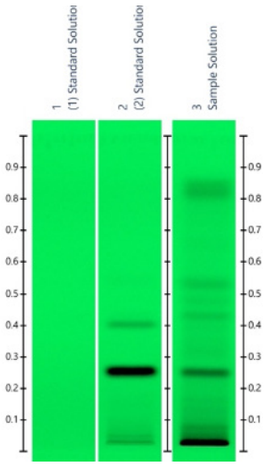
Plectranthus amboinicus (Loureiro) Sprengel (Fam. Labiatae/Lamiaceae)

Sample specimen solution:	The extraction method used is the same as the *Sample Solution* specified in the individual monograph
Standard specimen solution:	2.0 mg/mL of Carvacrol RS in methanol
Application volume:	0.2 µL sprayed as 9-mm bands
Developing solvent:	A mixture of hexane, ethyl acetate, and glacial acetic acid (1:1:0.05)
Spray reagent:	Anisaldehyde-sulfuric acid reagent

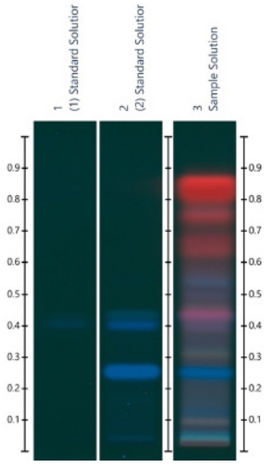
Underivatized



White Light

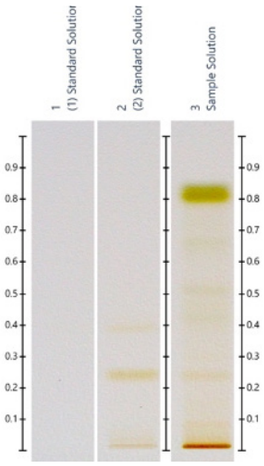


UV-254

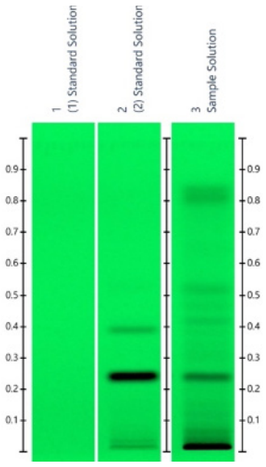


UV-366

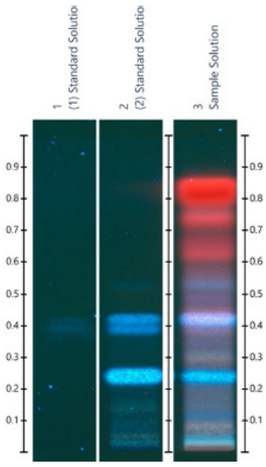
Derivatized



White Light



UV-254



UV-366

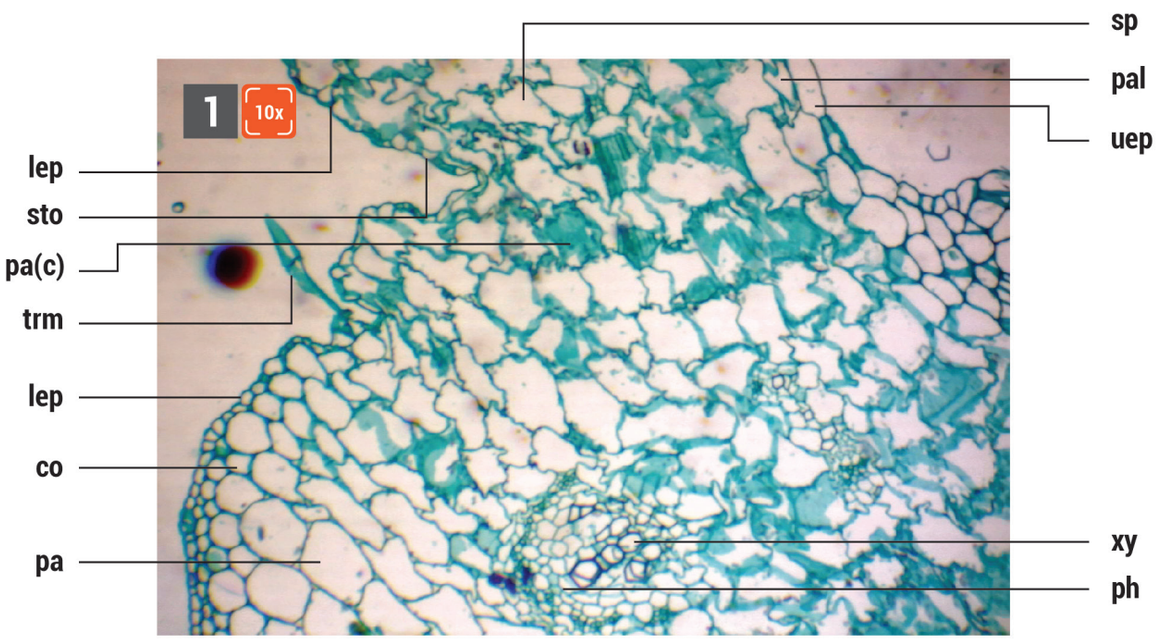
Plectranthi Amboinici Folium
Oregano Leaf
Plectranthi Amboinici Folium Pulveratum
Powdered Oregano Leaf
Reference HPTLC

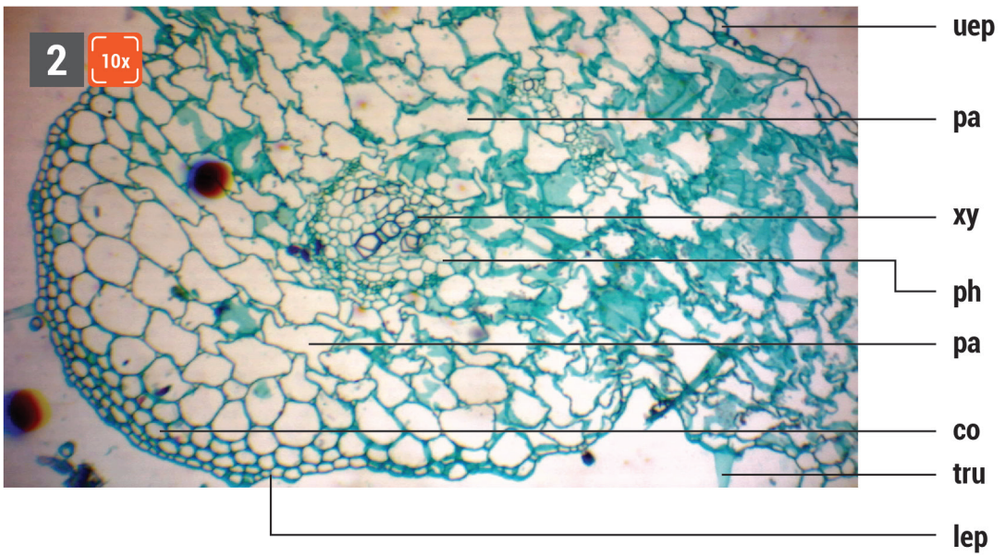
Table 6.6. Retention Factors (R_f) and Colors of Major Bands in Sample Solution

R_f VALUE	UNDERIVATIZED			DERIVATIZED		
	NORMAL LIGHT	UV-254	UV-366	NORMAL LIGHT	UV-254	UV-366
0.65*		Black	Red		Black	Red
0.61	Green		Blue/White	Brown		Blue/White
0.55						White
0.51	Yellow		Red	Green		
0.44			Red			Red
0.41	Yellow		Red	Dark Yellow		Red
0.38	Brown/ Yellow	Black	Red	Green	Black	Red
0.27		Black	Red		Black	Red
0.25			Red			Red

**Rf* value of Carvacrol - reference standard

PLECTRANTHI AMBOINICI FOLIUM | *Oregano Leaf*
MICROSCOPIC EXAMINATION





1. Cross Section of the Leaf Blade and Midrib

2. Cross Section of the Leaf Midrib

uep: upper epidermis; **pal:** palisade parenchyma; **sp:** spongy parenchyma; **lep:** lower epidermis; **sto:** stoma; **pa(c):** parenchyma cells with chloroplast; **trm:** multicellular trichome; **co:** collenchyma; **xy:** xylem; **ph:** phloem; **pa:** parenchyma; **tru:** unicellular trichome

SENNAE ALATAE FOLIUM | *Akapulko Leaf*

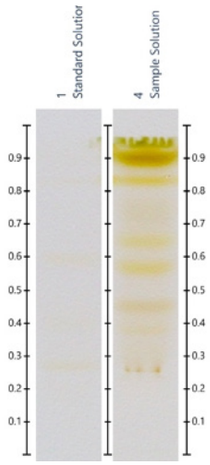
SCIENTIFIC NAME: Senna alata (L.) Roxb.
COMMON NAME: Akapulko
FAMILY: Fabaceae
HABITAT: Settled areas at low and medium altitudes
COLLECTION:
Place: Region 5, Arntic, Sablayon, Ligao, Albay
Collector: Pharmacopoeia Agri. Team
Date: 07/15/21
Certified by: Museum of Natural History, UPLB
Date: 01/19/22

Sennae Alatae Folium
***Akapulko* Leaf**
Sennae Alatae Folium Pulveratum
Powdered *Akapulko* Leaf
Reference HPTLC

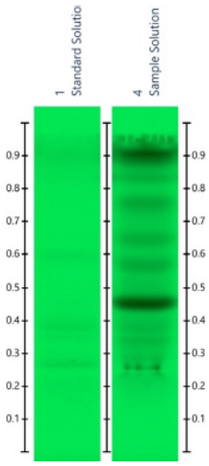
***Senna alata* (Linné) Roxburgh (Fam. Leguminosae/Fabaceae)**

Sample specimen solution:	The extraction method used is the same as the *Sample Solution* specified in the individual monograph
Standard specimen solution:	2.0 mg/mL of Senna Leaves RS in methanol
Application volume:	0.2 µL sprayed as 9-mm bands
Developing solvent:	A mixture of propanol, ethyl acetate, water, and glacial acetic acid (30:50:30:3)
Spray reagent:	20% nitric acid followed by 50g/L potassium hydroxide in 50% ethanol

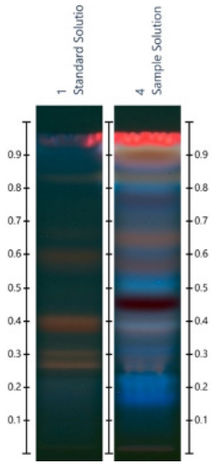
Underivatized



White Light

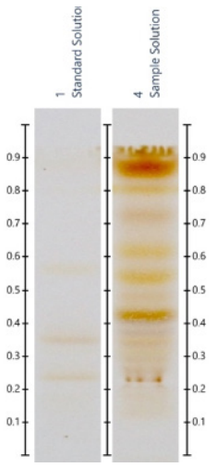


UV-254

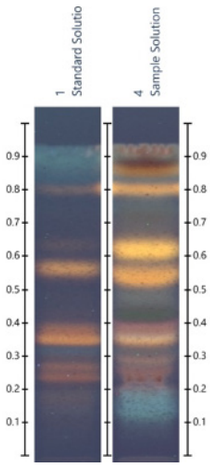


UV-366

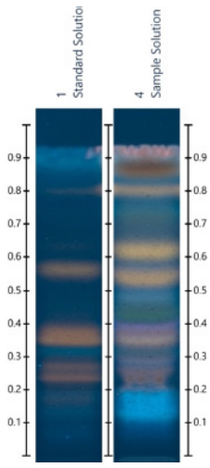
Derivatized



White Light



UV-254



UV-366

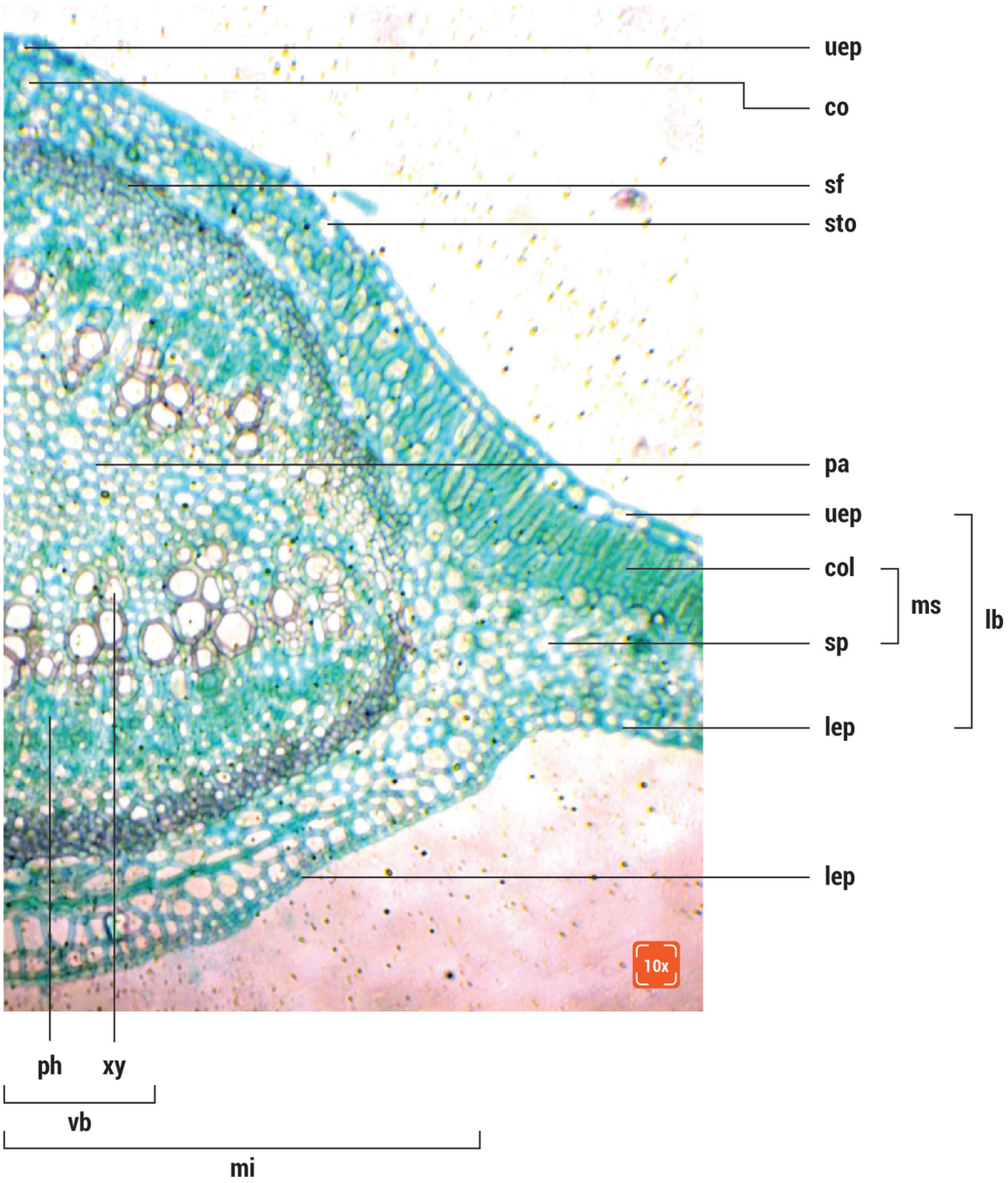
Sennae Alatae Folium
***Akapulko* Leaf**
Sennae Alatae Folium Pulveratum
Powdered *Akapulko* Leaf
Reference HPTLC

Table 6.7. Retention Factors (R_f) and Colors of Major Bands in Sample Solution

R_f VALUE	UNDERIVATIZED			DERIVATIZED		
	NORMAL LIGHT	UV-254	UV-366	NORMAL LIGHT	UV-254	UV-366
0.89	Yellow	Black		Dark Yellow		
0.83	Yellow	Black		Dark Yellow		
0.80*			Blue		Yellow	Dark Yellow
0.74		Black				
0.64	Yellow	Black		Dark Yellow		
0.60*					Yellow	Dark Yellow
0.55*	Yellow	Black		Dark Yellow	Yellow	Dark Yellow
0.49			Blue			
0.44	Light brown	Black		Brown		
0.43			Magenta			
0.41					Green	Green
0.39			Orange			
0.37*					Orange	Dark Yellow
0.34*					Yellow	Dark Yellow
0.25*			Orange		Dark Yellow	Dark Yellow

*R_f value of Sennosides from Senna Leaves - reference standard

SENNAE ALATAE FOLIUM | *Akapulko Leaf*
MICROSCOPIC EXAMINATION



Cross Section of the Leaf Blade and Midrib

uep: upper epidermis; **co:** collenchyma; **sf:** sclerenchyma fiber; **sto:** stoma; **pa:** parenchyma; **ph:** phloem; **xy:** xylem; **vb:** vascular bundle; **col:** columnar palisade parenchyma; **sp:** spongy cells; **ms:** mesophyll; **lep:** lower epidermis; **lb:** leaf blade; **mi:** midrib

VITICIS NEGUNDONIS FOLIUM | *Lagundi Leaf*

SCIENTIFIC NAME: Vitex negundo L.
COMMON NAME: Lagundi
FAMILY: Lamiaceae
HABITAT: At low and medium altitudes, in thickets and waste places
COLLECTION:
Place: Region 4A, UP Biotech, Los Baños, Laguna
Collector: Pharmacopoeia Agri. Team
Date: 5/23/21
Certified by: Museum of Natural History, UPLB
Date: 01/19/22

Viticis Negundonis Folium
***Lagundi* Leaf**
Viticis Negundonis Folium Pulveratum
Powdered *Lagundi* Leaf
Reference HPTLC

***Vitex negundo* Linné (Fam. Verbenaceae)**

Sample specimen solution: The extraction method used is the same as the *Sample Solution* specified in the individual monograph

Standard specimen solution: (1) 2.0 mg/mL of 3,5-dihydroxybenzoic acid in methanol

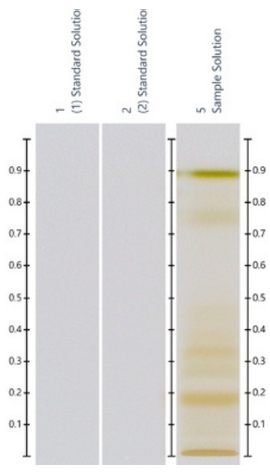
(2) 2.0 mg/mL of Negundoside RS dissolved in methanol

Application volume: 0.2 µL sprayed as 9-mm bands

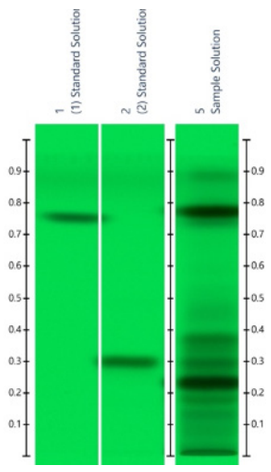
Developing solvent: A mixture of ethyl acetate, glacial acetic acid, and water (80:10:5)

Spray reagent: Anisaldehyde-sulfuric acid reagent

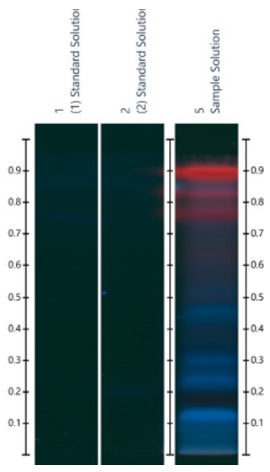
Underivatized



White Light

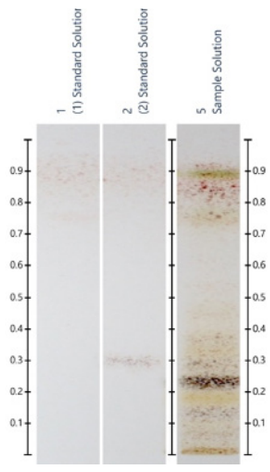


UV-254

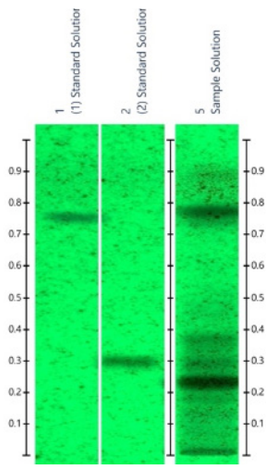


UV-366

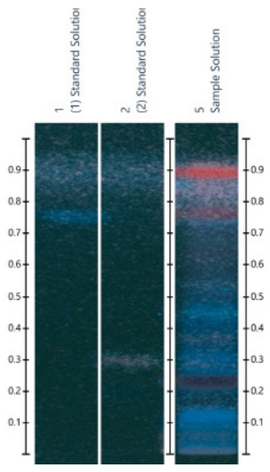
Derivatized



White Light



UV-254



UV-366

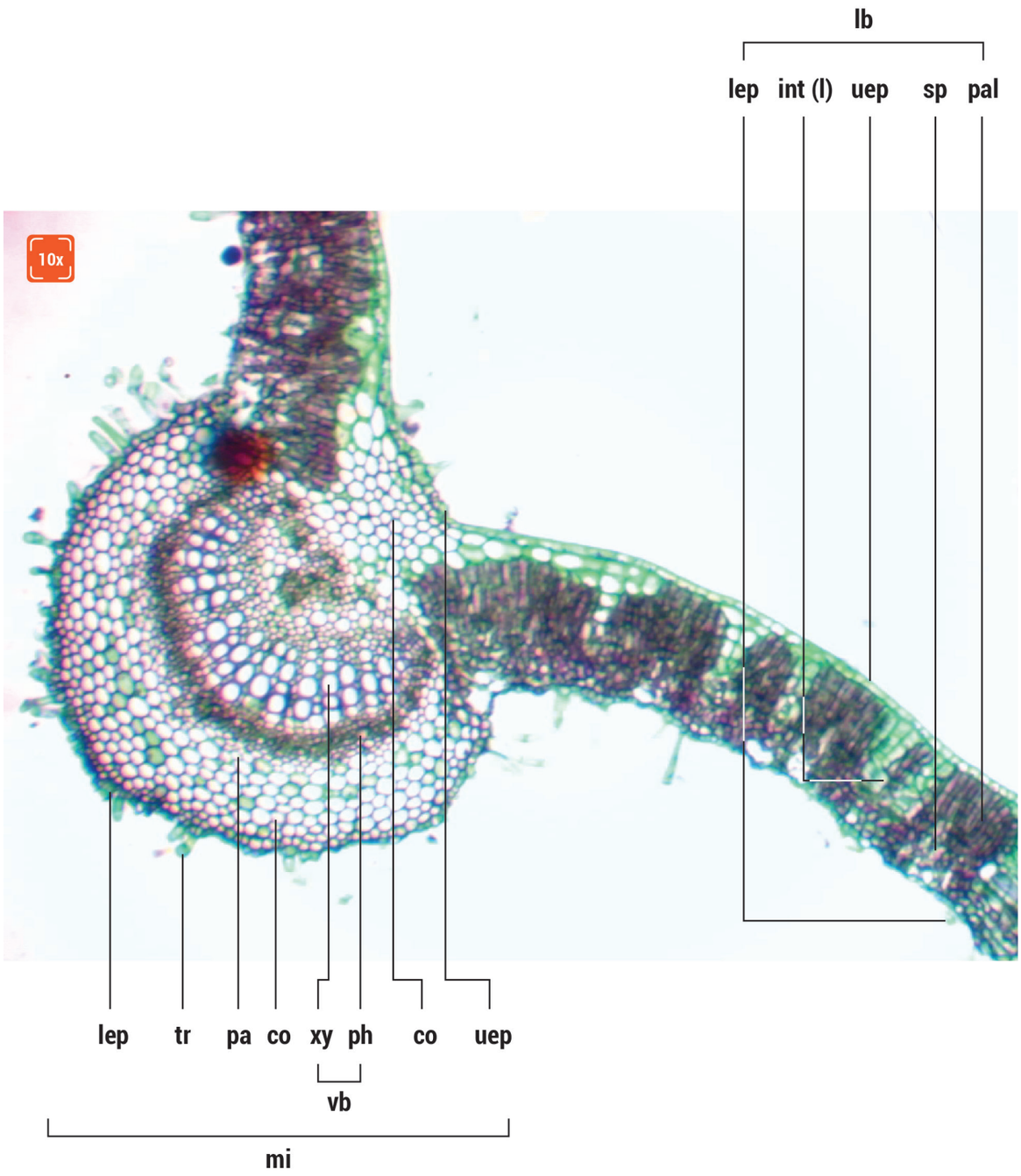
Viticis Negundonis Folium
***Lagundi* Leaf**
Viticis Negundonis Folium Pulveratum
Powdered *Lagundi* Leaf
Reference HPTLC

Table 6.8. Retention Factors (R_f) and Colors of Major Bands in Sample Solution

R_f VALUE	UNDERIVATIZED			DERIVATIZED		
	NORMAL LIGHT	UV-254	UV-366	NORMAL LIGHT	UV-254	UV-366
0.88	Green		Red	Green		Red
0.84				Brown		
0.81			Red			Red
0.76		Black	Red		Black	Red
0.73*		Black		Green	Black	Blue
0.43			Blue			Blue
0.35		Black			Black	
0.31	Dark yellow					
0.28**		Black		Brown	Black	Pink
0.22		Black	Blue	Black	Black	Magenta
0.17	Dark Yellow		Violet	Yellow		
0.11			Blue	Brown		Blue

*R_f value of 3,5-dihydroxybenzoic acid - reference standard
**R_f value of Negundoside - reference standard

VITICIS NEGUNDONIS FOLIUM | *Lagundi Leaf*
MICROSCOPIC EXAMINATION



Cross Section of the Leaf Blade and Midrib

lep: lower epidermis; **tr:** trichome; **pa:** parenchyma; **co:** collenchyma; **xy:** xylem; **ph:** phloem; **vb:** vascular bundle; **uep:** upper epidermis; **mi:** midrib; **pal:** palisade parenchyma; **sp:** spongy parenchyma; **lb:** leaf blade; **int (l):** large intracellular space

ZINGIBERIS OFFICINALIS RHIZOMA | *Luya Rhizome*

SCIENTIFIC NAME: Zingiber officinale Roscoe
COMMON NAME: Luya
FAMILY: Zingiberaceae
HABITAT: Moist, humid, partly shaded tropical and subtropical forests of Southeast Asia; widely cultivated in the Philippines
COLLECTION:
Place: Region 4A, UP Biotech, Los Baños, Laguna
Collector: Pharmacopoeia Agri. Team
Date: 03/04/21
Certified by: Museum of Natural History, UPLB
Date:

Zingiberis Officinalis Rhizome
***Luya* Rhizome**
Zingiberis Officinalis Rhizome Pulveratum
Powdered *Luya* Rhizome
Reference HPTLC

***Zingiber officinale* Rosc (Fam. Zingiberaceae)**

Sample specimen solution: The extraction method used is the same as the *Sample Solution* specified in the individual monograph

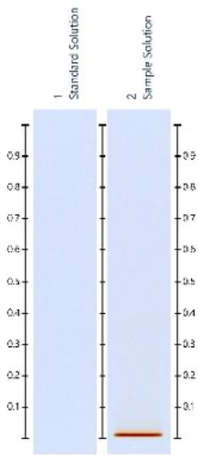
Standard specimen solution: 1 mg/mL of Gingerol in methanol

Application volume: 0.2 µL sprayed as 9-mm bands

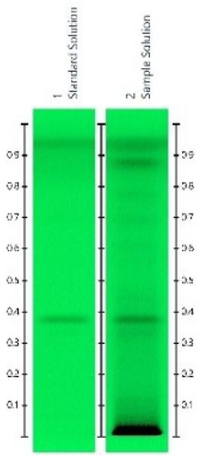
Developing solvent: A mixture of hexane, ethyl acetate, formic acid (62.19:37.31:0.5)

Spray reagent: Anisaldehyde-sulfuric acid reagent

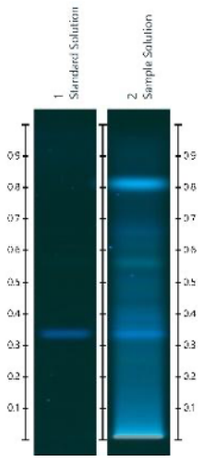
Underivatized



White Light

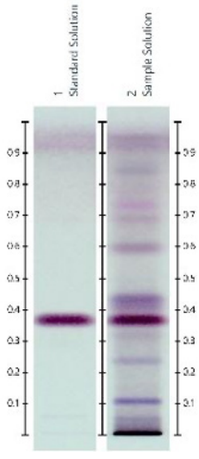


UV-254

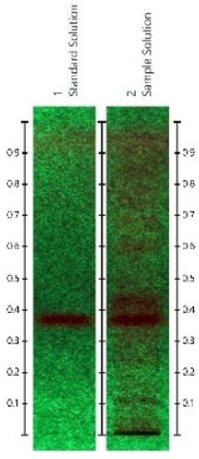


UV-366

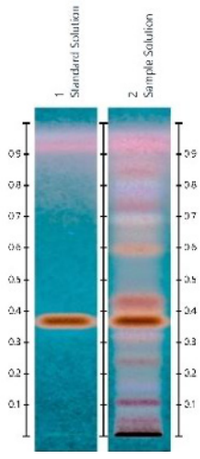
Derivatized



White Light



UV-254



UV-366

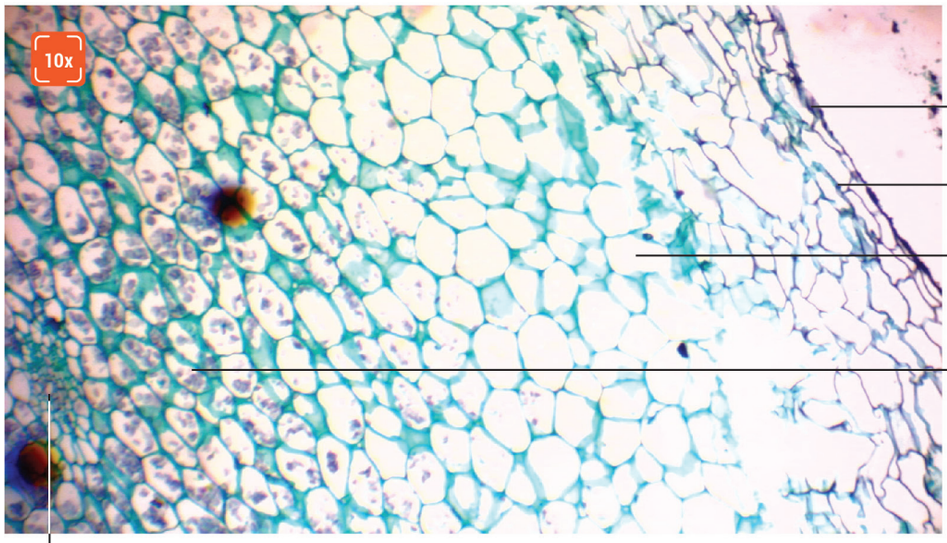
Zingiberis Officinalis Rhizome
***Luya* Rhizome**
Zingiberis Officinalis Rhizome Pulveratum
Powdered *Luya* Rhizome
Reference HPTLC

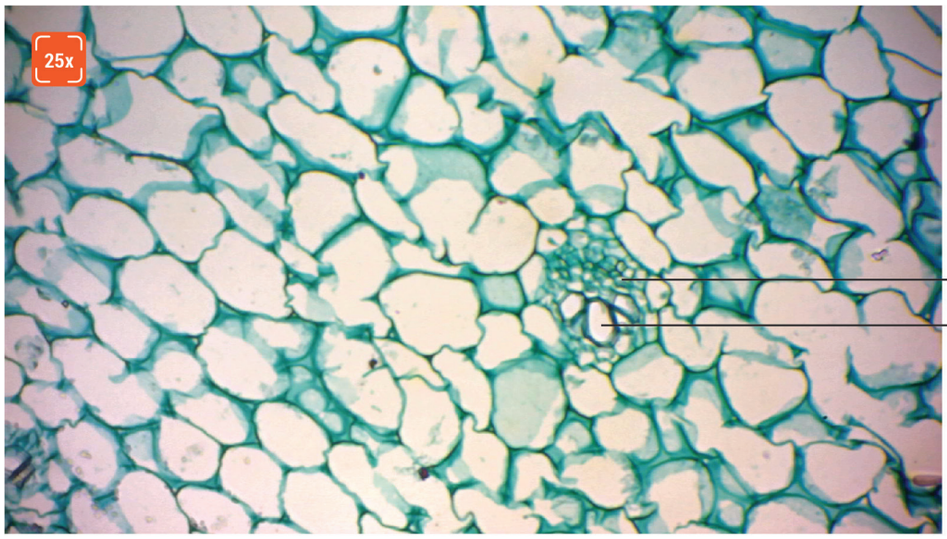
Table 6.9. Retention Factors (R_f) and Colors of Major Bands in Sample Solution

R_f VALUE	UNDERIVATIZED			DERIVATIZED		
	NORMAL LIGHT	UV-254	UV-366	NORMAL LIGHT	UV-254	UV-366
0.88		Black				
0.81			Blue			
0.78						Pink
0.75						Red
0.71		Black	Blue	Purple	Brown	
0.69				Purple		Brown
0.6				Purple		
0.56			Blue			
0.43				Purple	Brown	Brown
0.37*		Black	Blue	Violet	Brown	
0.34						Brown
0.32				Purple		Brown
0.24				Purple		Violet
0.1				Indigo	Brown	Violet

*R_f value of Gingerol – reference standard

ZINGIBERIS OFFICINALIS RHIZOMA | *Luya Rhizome*
MICROSCOPIC EXAMINATION





1. Cross Section of the Rhizome

2. Cross Section of the Rhizome showing the Vascular Bundle

ep: epidermis; **hy:** hypodermis; **pa:** parenchyma; **st:** starch grain; **vb:** vascular bundle; **ph:** phloem; **xy:** xylem

MEDICINAL PLANTS

ALII SATIVI BULBUS | *Bawang Cloves*

SCIENTIFIC NAME: Allium sativum L.
COMMON NAME: Bawang
FAMILY: Liliaceae
HABITAT: Cultivated all over the world in warm, moderate climate
COLLECTION
Place: Region 1, Burgos, Ilocos Norte
Collector: Pharmacopoeia Agri. Team
Date: 02/01/22
Certified by: Museum of Natural History, UPLB
Date:

Allii Sativi Bulbus
Bawang Cloves
Allii Sativi Bulbus Pulveratum
Powdered _Bawang_ Cloves
Reference HPTLC

Allium sativum Linn (Fam. Alliaceae)

Sample specimen solution: The extraction method used is the same as the _Sample Solution_ specified in the individual monograph

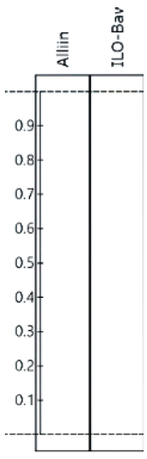
Standard specimen solution: 1 mg/mL of Alliin in methanol

Application volume: 0.2 µL sprayed as 9-mm bands

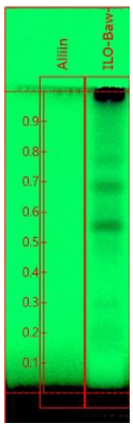
Developing solvent: A mixture of butanol, acetic acid, water, chloroform (47.63:22.13:19.83:10.42)

Spray reagent: Ninhydrin reagent

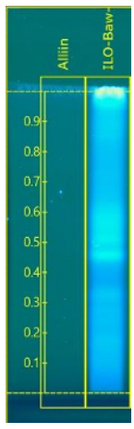
Underivatized



White Light

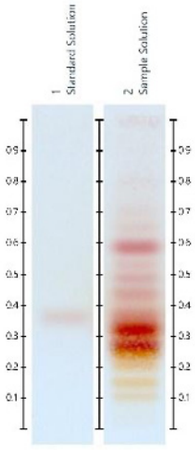


UV-254

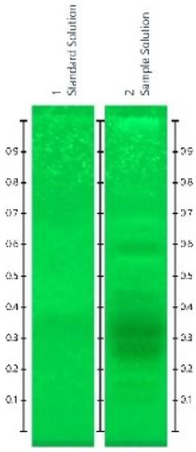


UV-366

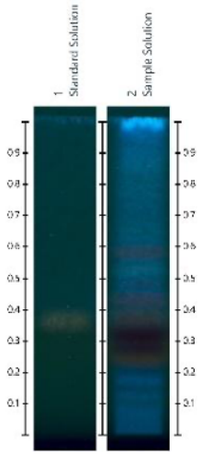
Derivatized



White Light



UV-254



UV-366

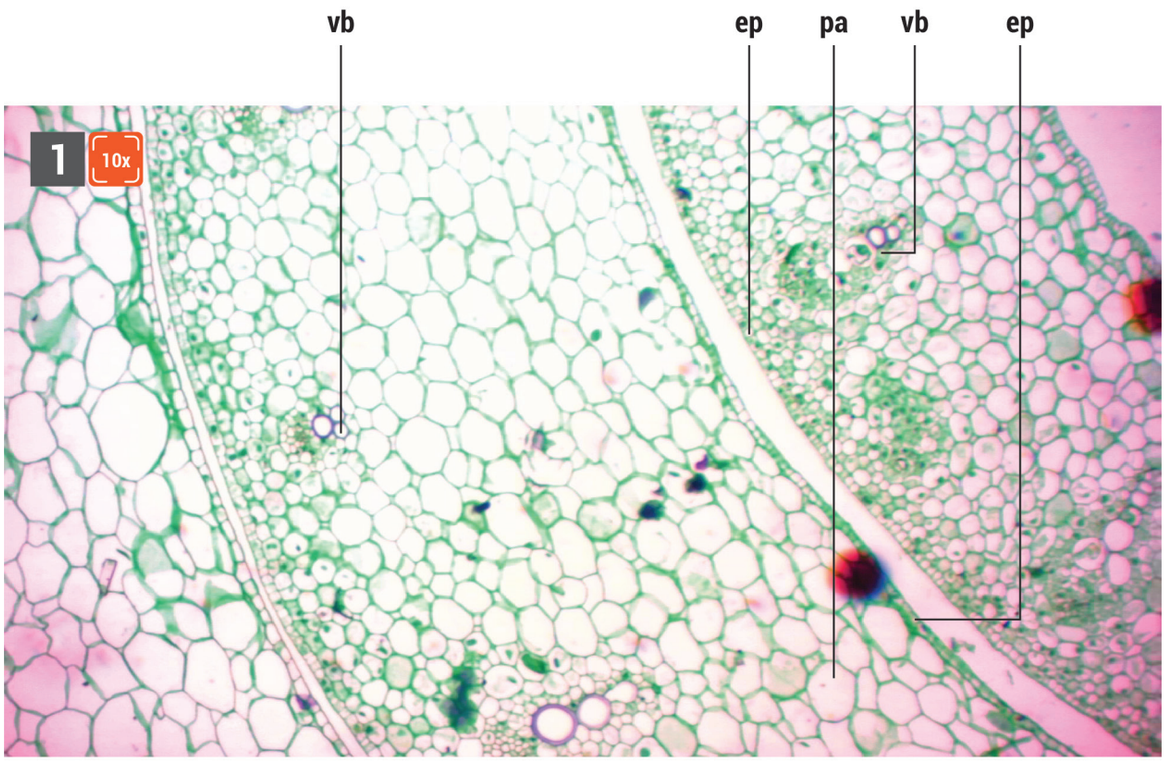
Blumeae Balsamiferae Folium
***Sambong* Leaf**
Blumeae Balsamiferae Folium Pulveratum
Powdered *Sambong* Leaf
Reference HPTLC

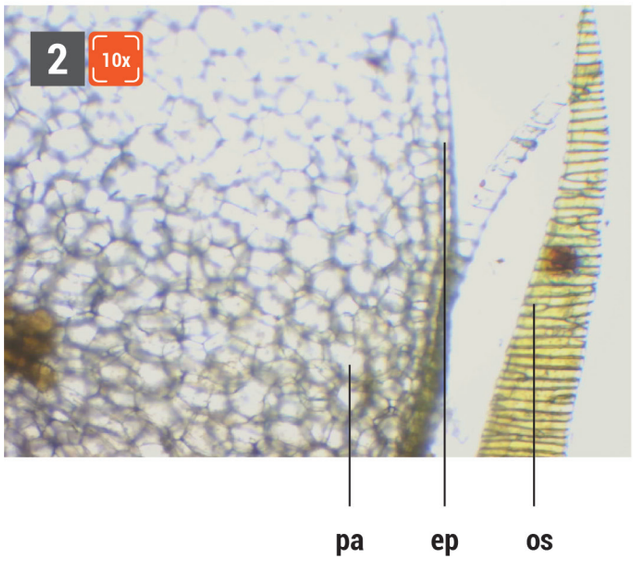
Table 6.10. Retention Factors (R_f) and Colors of Major Bands in Sample Solution

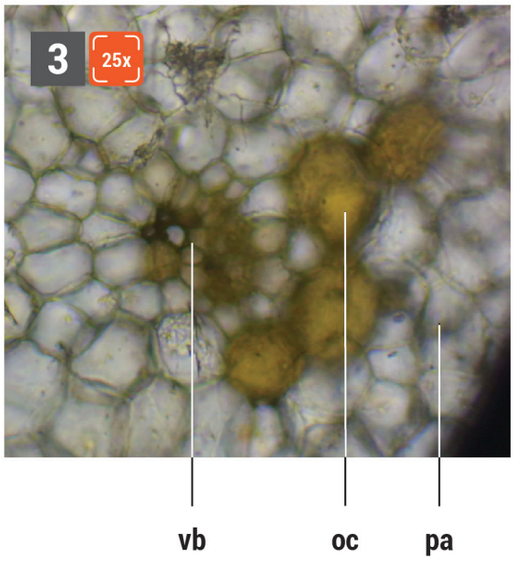
R_f VALUE	UNDERIVATIZED			DERIVATIZED		
	NORMAL LIGHT	UV-254	UV-366	NORMAL LIGHT	UV-254	UV-366
0.59					Black	Red
0.54		Black		Pink	Black	Black
0.52						Black
0.49		Black		Pink	Black	Brown
0.43				Pink	Black	
0.37*				Pink	Black	Yellow
0.32				Red Orange	Black	Brown
0.26				Orange	Black	Brown
0.21				Orange	Black	
0.15				Orange	Black	Brown
0.11				Orange	Black	Brown

*R_f value of Alliin – reference standard

ALII SATIVI BULBUS | *Bawang Cloves*
MICROSCOPIC EXAMINATION







1. Cross Section of the Clove

2. Cross Section of the Clove with Outer Scale

3. Cross Section of the Clove showing the Oil Cell

vb: vascular bundle; ep: epidermis; pa: parenchyma; os: outer scale; oc: oil cell

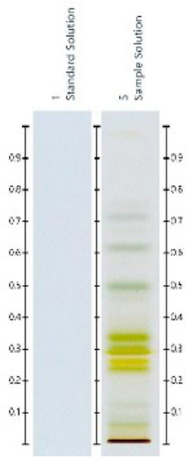
CASSIAE FISTULAE FOLIUM | *Kanya Pistula Leaf*

Cassiae Fistulae Folium
***Kanya Pistula* Leaf**
Cassiae Fistulae Folium Pulveratum
Powdered *Kanya Pistula* Leaf
Reference HPTLC

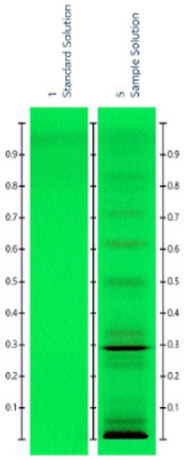
***Cassia fistula* Linn (Fam. Caesalpiniaceae)**

Sample specimen solution:	The extraction method used is the same as the *Sample Solution* specified in the individual monograph
Standard specimen solution:	1 mg/mL of β-Sitosterol in methanol
Application volume:	0.2 µL sprayed as 9-mm bands
Developing solvent:	A mixture of hexane, ethyl acetate, formic acid (66.45:33.22:0.33)
Spray reagent:	Anisaldehyde-sulfuric acid reagent

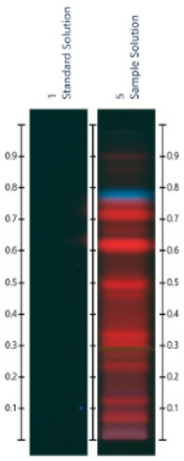
Underivatized



White Light

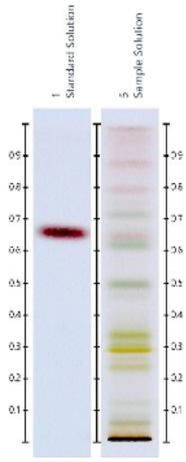


UV-254

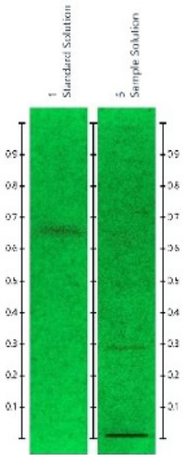


UV-366

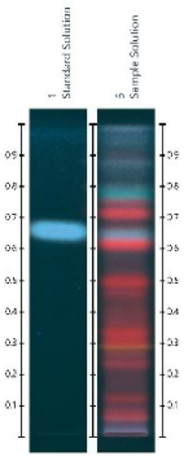
Derivatized



White Light



UV-254



UV-366

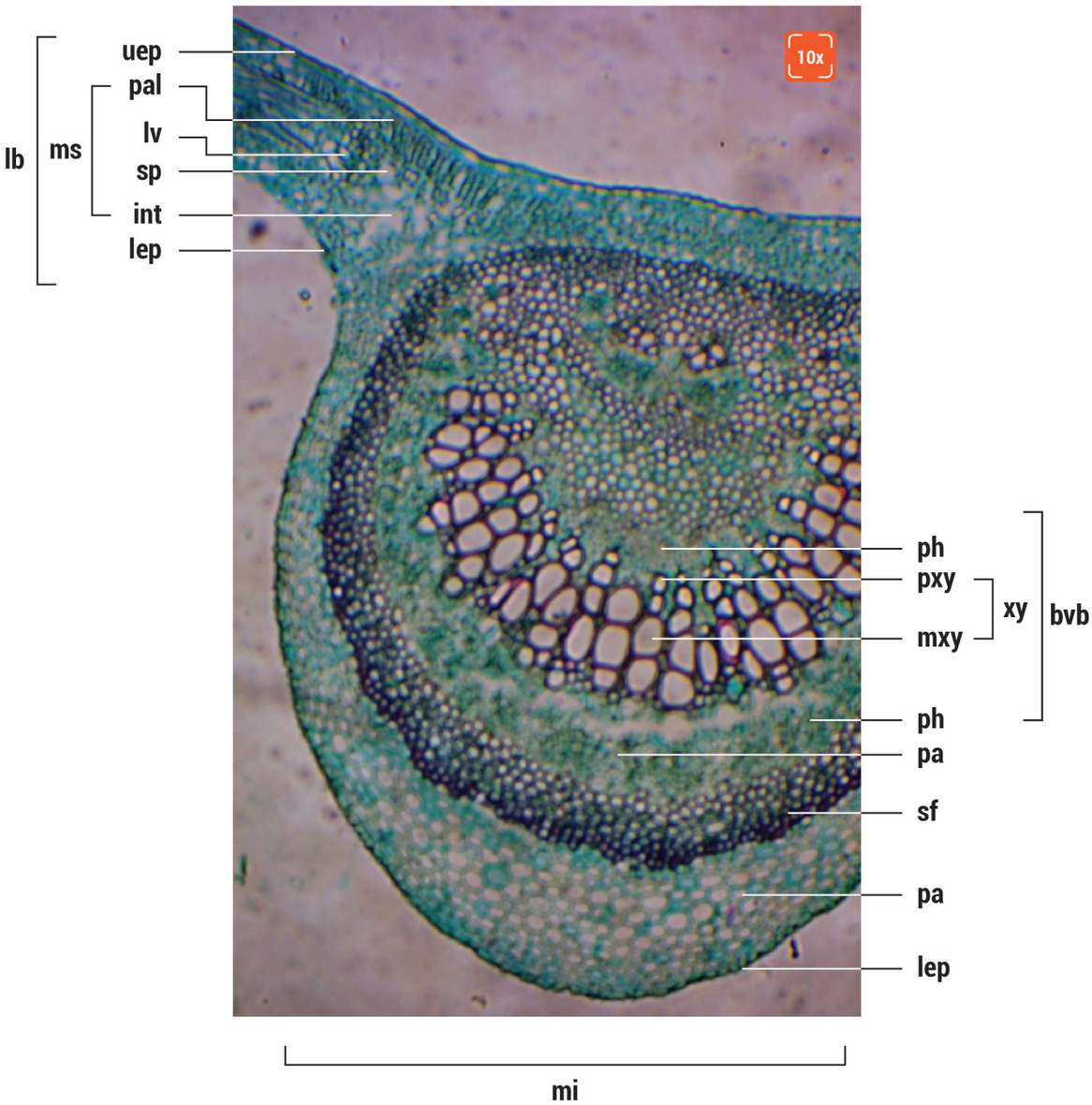
Cassiae Fistulae Folium
***Kanya Pistula* Leaf**
Cassiae Fistulae Folium Pulveratum
Powdered *Kanya Pistula* Leaf
Reference HPTLC

Table 6.11. Retention Factors (R_f) and Colors of Major Bands in Sample Solution

R_f VALUE	UNDERIVATIZED			DERIVATIZED		
	NORMAL LIGHT	UV-254	UV-366	NORMAL LIGHT	UV-254	UV-366
0.78						Blue
0.71			Red			Red
0.65*				Red	Black	Blue
0.62	Green	Black	Red	Green		Red
0.49	Green	Black	Red	Green		Red
0.34	Yellow Green	Black	Red	Green		Red
0.29	Yellow Green	Black	Brown	Yellow Green	Black	Brown
0.26	Yellow	Black	Red	Green		Red

*R_f value of β-sitosterol – reference standard

CASSIAE FISTULAE FOLIUM | *Kanya Pistula Leaf*
MICROSCOPIC EXAMINATION



Cross Section of the Leaf Blade and Midrib

uep: upper epidermis; **pal:** palisade parenchyma; **lv:** lateral vein; **sp:** spongy parenchyma; **int:** interceullar space; **ms:** mesophyll; **lep:** lower epidermis; **lb:** leaf blade; **ph:** phloem; **pxy:** protoxylem; **mxy:** metaxylem; **xy:** xylem; **bvb:** bicollateral vascular bundle; **pa:** parenchyma; **sf:** sclerenchyma fiber; **lep:** lower epidermis

CURCUMAE LONGAE RHIZOMA | *Luyang Dilaw Rhizomes*

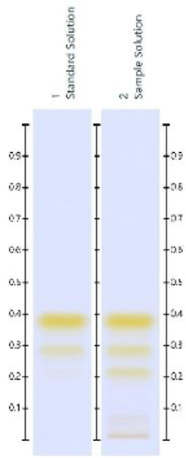
SCIENTIFIC NAME: Curcuma longa L.
COMMON NAME: Luyang dilaw
FAMILY: Zingiberaceae
HABITAT: Open waste places or secondary forests; cultivated in the Philippines
COLLECTION:
Place: Region 2, CVHPP, Cagayan, Tuguegarao
Collector: Pharmacopoeia Agri. Team
Date: 02/18/22
Certified by: Museum of Natural History, UPLB
Date:

Curcumae Longae Rhizome
***Luyang Dilaw* Rhizome**
Curcumae Longae Rhizome Pulveratum
Powdered *Luyang Dilaw* Rhizome
Reference HPTLC

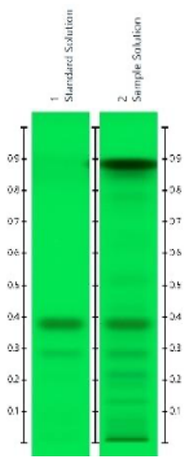
***Curcuma longa* Linn (Fam. Zingiberaceae)**

Sample specimen solution:	The extraction method used is the same as the *Sample Solution* specified in the individual monograph
Standard specimen solution:	1 mg/mL of Curcumin in methanol
Application volume:	0.2 µL sprayed as 9-mm bands
Developing solvent:	A mixture of chloroform, hexane, acetone, formic acid (69.82:19.95:9.98:0.25)
Spray reagent:	Anisaldehyde-sulfuric acid reagent

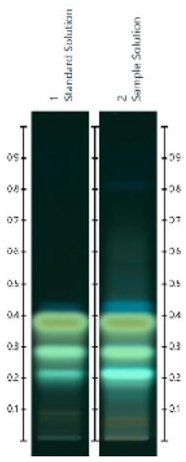
Underivatized



White Light

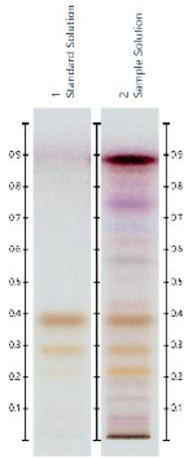


UV-254

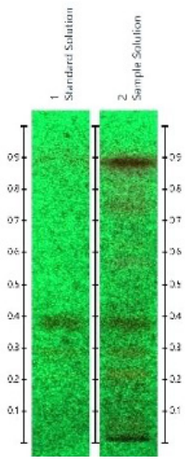


UV-366

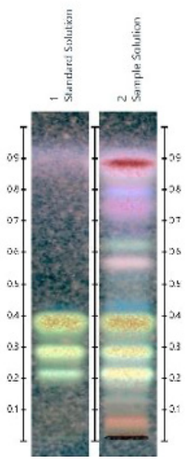
Derivatized



White Light



UV-254



UV-366

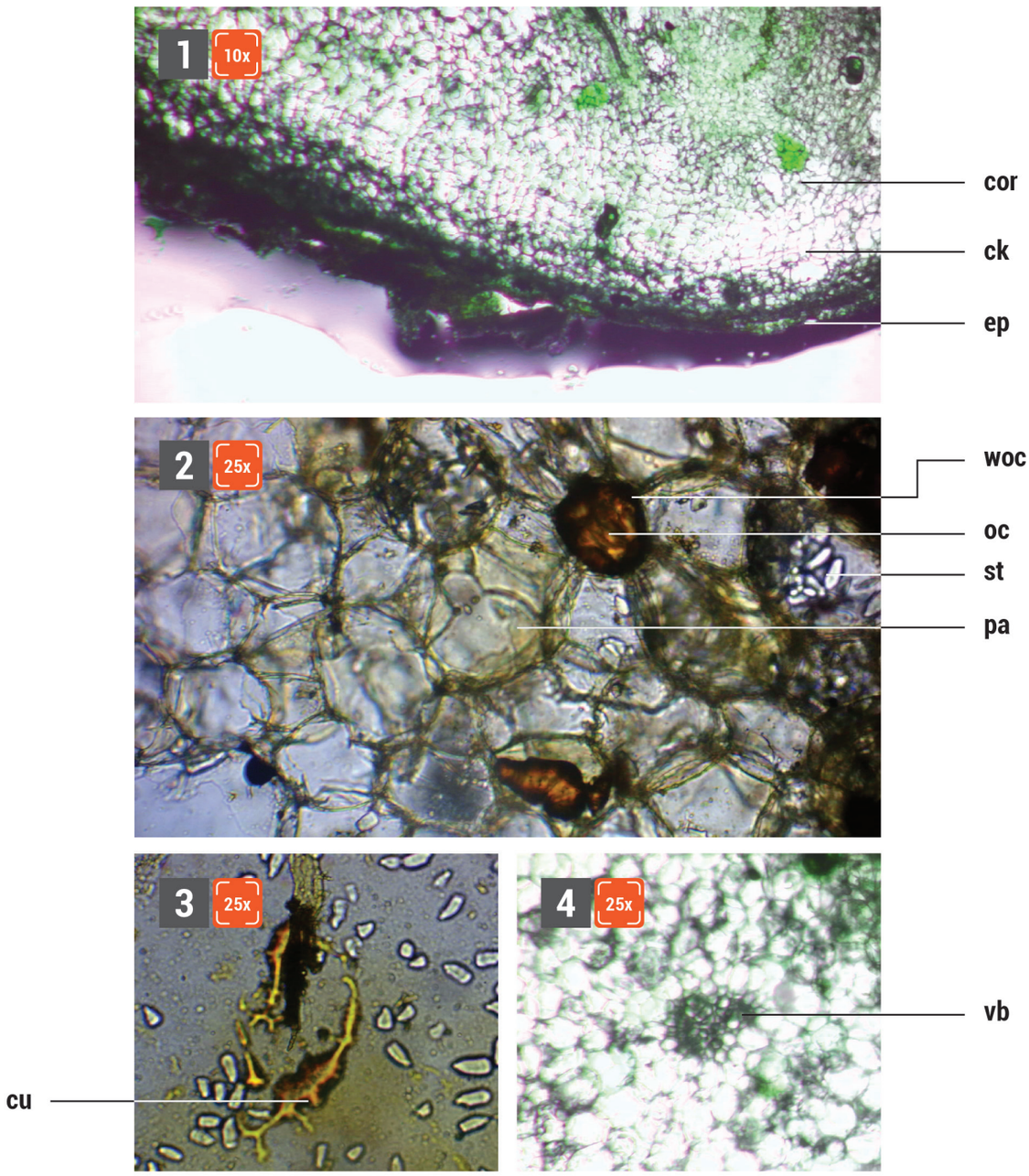
Curcumae Longae Rhizome
***Luyang Dilaw* Rhizome**
Curcumae Longae Rhizome Pulveratum
Powdered *Luyang Dilaw* Rhizome
Reference HPTLC

Table 6.12. Retention Factors (R_f) and Colors of Major Bands in Sample Solution

R_f VALUE	UNDERIVATIZED			DERIVATIZED		
	NORMAL LIGHT	UV-254	UV-366	NORMAL LIGHT	UV-254	UV-366
0.89		Black		Purple	Brown	Violet
0.85		Black		Purple		Gray
0.79						Blue
0.74		Black		Violet		Pink
0.68				Blue		
0.63		Black		Purple		Yellow
0.52		Black				
0.5			Blue			
0.38*	Yellow	Black	Light yellow	Brown	Dark Brown	Yellow
0.3*	Yellow	Black	Light yellow	Brown	Brown	Yellow
0.23*	Yellow	Black	Light yellow	Orange brown	Brown	Yellow
0.19		Black				

*R_f value of Curcumin – reference standard

CURCUMAE LONGAE RHIZOMA | *Luyang Dilaw Rhizomes*
MICROSCOPIC EXAMINATION



1. Cross Section of the Rhizome

2. Cross Section of the Rhizome showing Oil Cells

3. Curcumin and Starch Grains in the Rhizome

4. Cross Section of the Rhizome showing the Vascular Bundle

cor: cortex; ck: cork; **ep:** epidermis; **woc:** subserized walls of oil cells; **oc:** oil cell; **st:** starch grain; **pa:** parenchyma; **cu:** curcumin; **vb:** vascular bundle

LEUCAENAE LEUCOCEPHALAE SEMEN | *Ipil-Ipil Seeds*

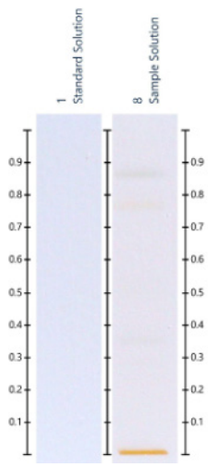
SCIENTIFIC NAME: Leucaena leucocephala (Lam.) de Wit
COMMON NAME: Ipil-ipil
FAMILY: Fabaceae
HABITAT: Settled areas at low and medium altitudes
COLLECTION
Place: Region 1, Aguilar, Pangasinan
Collector: Pharmacopoeia Agri. Team
Date: 07/26/21
Certified by: Museum of Natural History, UPLB
Date: 01/19/22

Leucaenae Leucocephalae Semen
***Ipil-ipil* Seeds**
Leucaenae Leucocephalae Semen Pulveratum
Powdered *Ipil-ipil* Seeds
Reference HPTLC

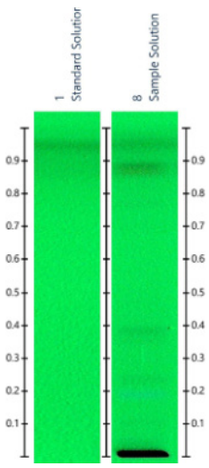
***Leucaenae leucocephalae* Lam (Fam. Fabaceae)**

Sample specimen solution:	The extraction method used is the same as the *Sample Solution* specified in the individual monograph
Standard specimen solution:	1 mg/mL of β-sitosterol in methanol
Application volume:	0.2 µL sprayed as 9-mm bands
Developing solvent:	A mixture of hexane, ethyl acetate, formic acid (41.80:22.86:2.44)
Spray reagent:	Anisaldehyde-sulfuric acid reagent

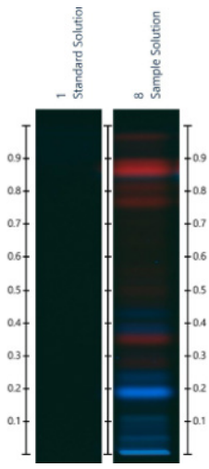
Underivatized



White Light

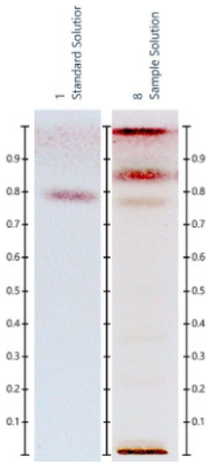


UV-254

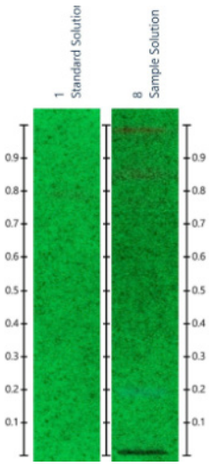


UV-366

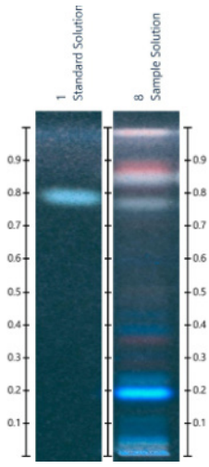
Derivatized



White Light



UV-254



UV-366

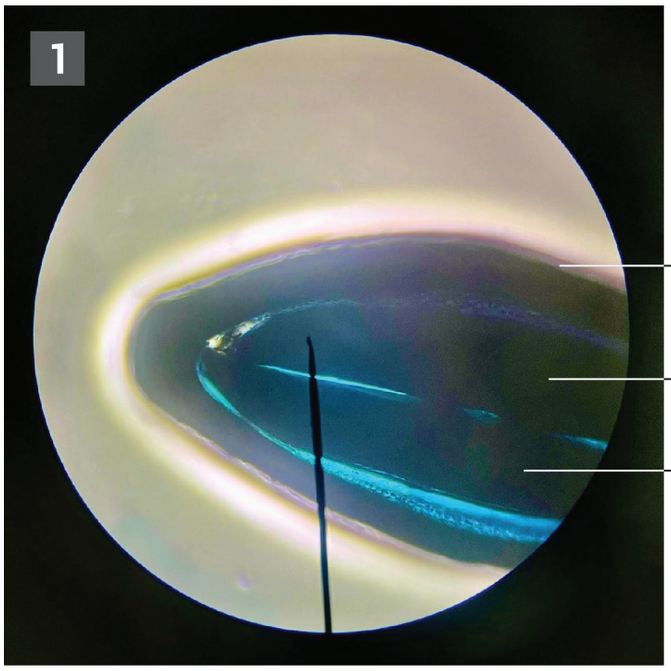
Leucaenae Leucocephalae Semen
***Ipil-ipil* Seeds**
Leucaenae Leucocephalae Semen Pulveratum
Powdered *Ipil-ipil* Seeds
Reference HPTLC

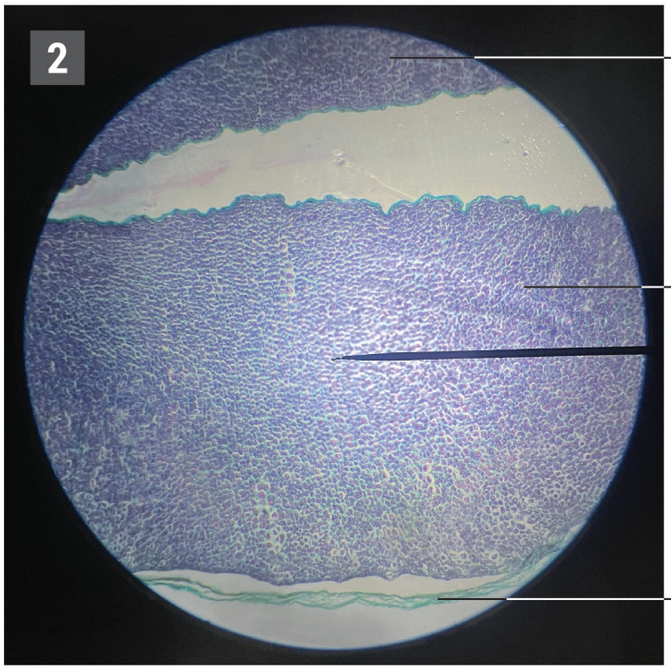
Table 6.13. Retention Factors (R_f) and Colors of Major Bands in Sample Solution

R_f VALUE	UNDERIVATIZED			DERIVATIZED		
	NORMAL LIGHT	UV-254	UV-366	NORMAL LIGHT	UV-254	UV-366
0.87		Black	Red	Red	Black	
0.85						White
0.82			Red			
0.77*	Yellow		Red	Red	Black	White
0.37		Black				
0.34	Green		Red	Green		Red
0.27			Red			
0.22			Blue	Red		Blue
0.18		Blue	Blue		Blue	Blue

*R_f value of β-sitosterol – reference standard

LEUCAENAE LEUCOCEPHALAE SEMEN | *Ipil-Ipil Seeds*
MICROSCOPIC EXAMINATION





1. Scanner View of the Cross Section of the Seed

2. Cross Section of the Seed

sd: seed coat; **cot:** cotyledon

MORINGAE OLEIFERAE FOLIUM | *Malunggay Leaf*

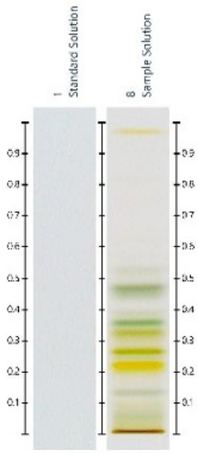
SCIENTIFIC NAME: Moringa oleifera Lam.
COMMON NAME: Malunggay
FAMILY: Moringaceae
HABITAT: Settled areas at low and medium altitudes, common in backyards as vegetable and border plant
COLLECTION:
Place: Region 4A, UP Biotech, Los Baños, laguna
Collector: Pharmacopoeia Agri. Team
Date: 09/21/21
Certified by: Museum of Natural History, UPLB
Date: 01/19/22

Moringae Oleiferae Folium
Malunggay Leaf
Moringae Oleiferae Folium Pulveratum
Powdered _Malunggay_ Leaf
Reference HPTLC

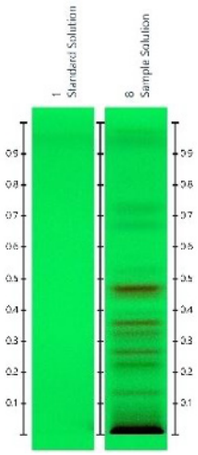
Moringa oleifera Lam (Fam. Moringaceae)

Sample specimen solution:	The extraction method used is the same as the _Sample Solution_ specified in the individual monograph
Standard specimen solution:	1 mg/mL of β-sitosterol in methanol
Application volume:	0.2 µL sprayed as 9-mm bands
Developing solvent:	A mixture of hexane, ethyl acetate, and formic acid (69.48:29.78:0.74)
Spray reagent:	Anisaldehyde-sulfuric acid reagent

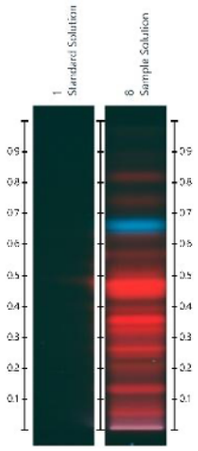
Underivatized



White Light

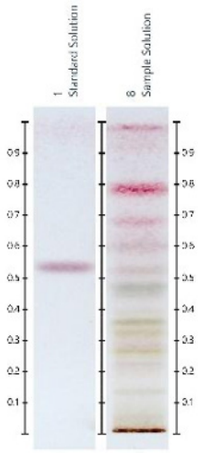


UV-254

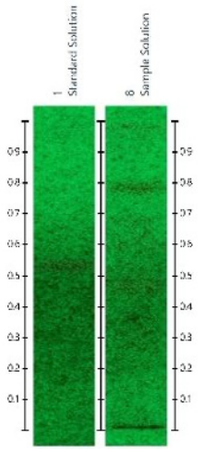


UV-366

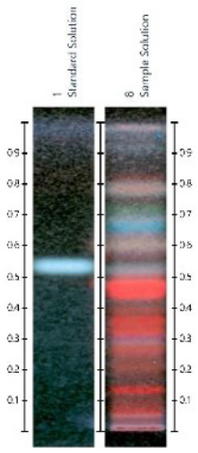
Derivatized



White Light



UV-254



UV-366

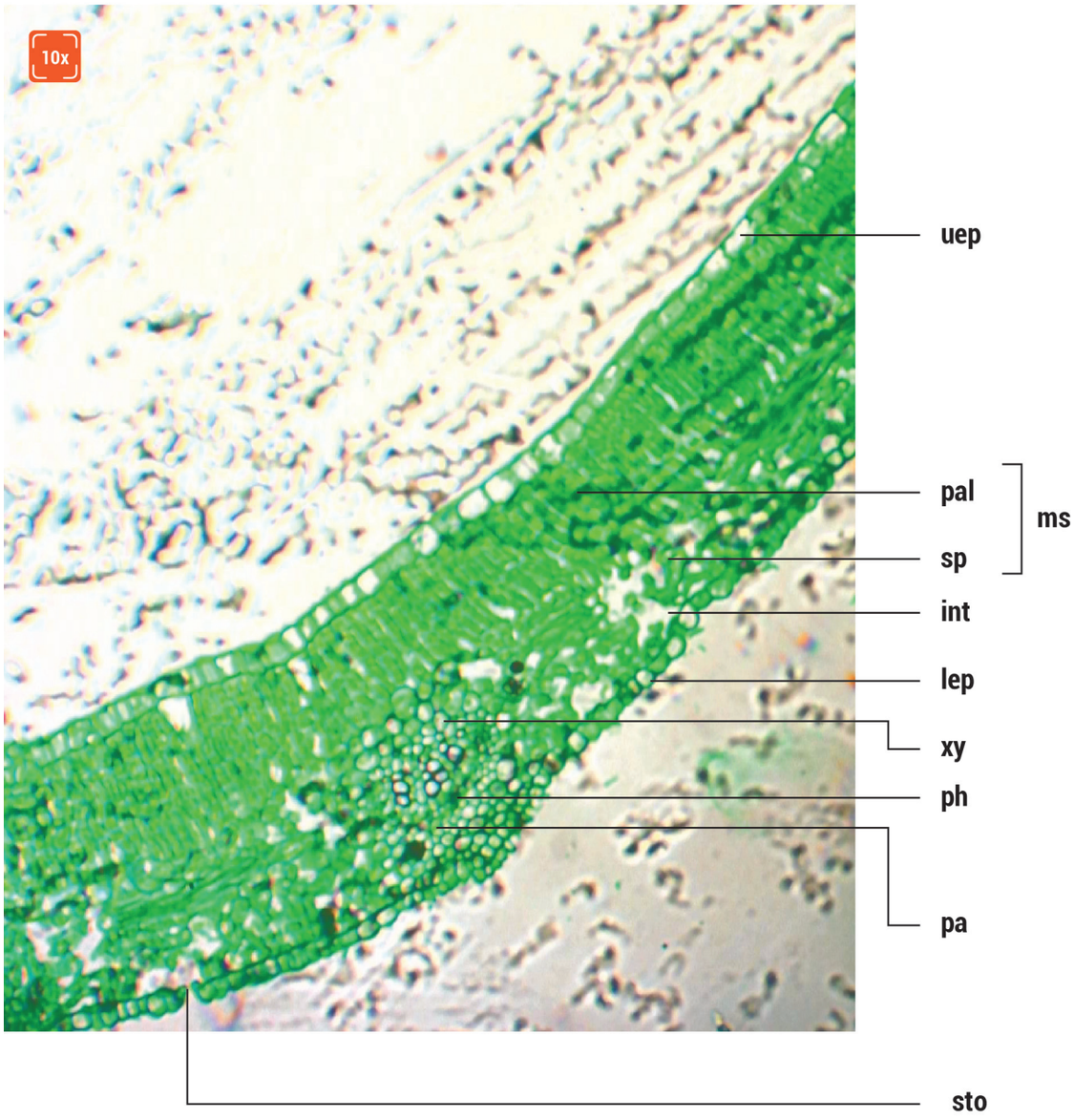
Moringae Oleiferae Folium
***Malunggay* Leaf**
Moringae Oleiferae Folium Pulveratum
Powdered *Malunggay* Leaf
Reference HPTLC

Table 6.14. Retention Factors (R_f) and Colors of Major Bands in Sample Solution

R_f VALUE	UNDERIVATIZED			DERIVATIZED		
	NORMAL LIGHT	UV-254	UV-366	NORMAL LIGHT	UV-254	UV-366
0.94				Red		
0.81			Red			
0.78			Red	Red	Black	Red
0.66		Black		Red		
0.57			Red			Red
0.53*				Red		Blue
0.52	Green	Black				
0.48	Green	Black	Red	Green		Red
0.40		Black	Red	Green		Red
0.37			Red	Green		Red
0.28	Green	Black	Red	Green		
0.23	Yellow					

*R_f value of β-sitosterol – reference standard

MORINGAE OLEIFERAE FOLIUM | *Malunggay Leaf*
MICROSCOPIC EXAMINATION



Cross Section of the Leaf

uep: upper epidermis; **pal:** palisade parenchyma; **sp:** spongy parenchyma; **ms:** mesophyll; **int:** intercellular space; **lep:** lower epidermis; **xy:** xylem; **ph:** phloem; **pa:** parenchyma; **sto:** stoma

PSIDII GUAJAVAE FOLIUM | *Guava Leaf*

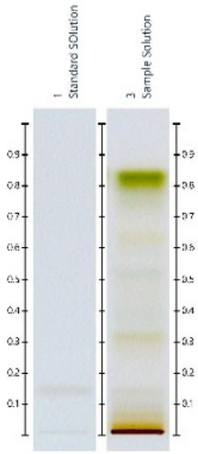
SCIENTIFIC NAME: Psidium guajava L.
COMMON NAME: Bayabas (with white mesocarp)
FAMILY: Myrtaceae
HABITAT: Settled areas and in thickets and secondary forests at low altitudes
COLLECTION:
Place: Region 4A, UP Biotech, Los Baños, Laguna
Collector: Pharmacopoeia Agri. Team
Date: 07/04/21
Certified by: Museum of Natural History, UPLB
Date: 01/19/22

Psidii Guajavae Folium
***Bayabas* Leaf**
Psidii Guajavae Folium Pulveratum
Powdered *Bayabas* Leaf
Reference HPTLC

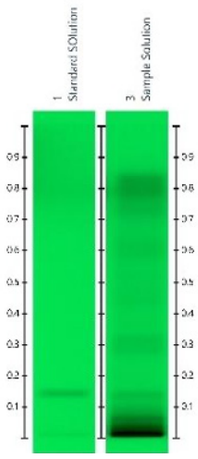
***Psidium guajava* Linn (Fam. Myrtaceae)**

Sample specimen solution:	The extraction method used is the same as the *Sample Solution* specified in the individual monograph
Standard specimen solution:	1 mg/mL of Gallic acid in methanol
Application volume:	0.2 µL sprayed as 9-mm bands
Developing solvent:	A mixture of chloroform, acetone, and formic acid (10:2:1)
Spray reagent:	Anisaldehyde-sulfuric acid reagent

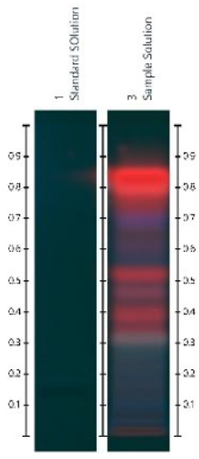
Underivatized



White Light

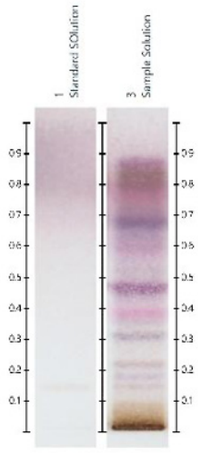


UV-254

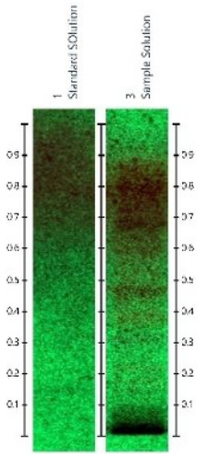


UV-366

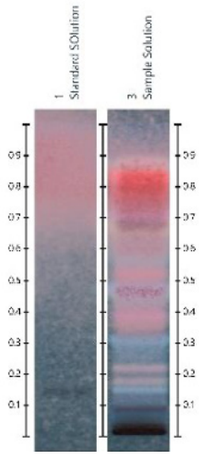
Derivatized



White Light



UV-254



UV-366

Psidii Guajavae Folium

***Bayabas* Leaf**

Psidii Guajavae Folium Pulveratum

Powdered *Bayabas* Leaf

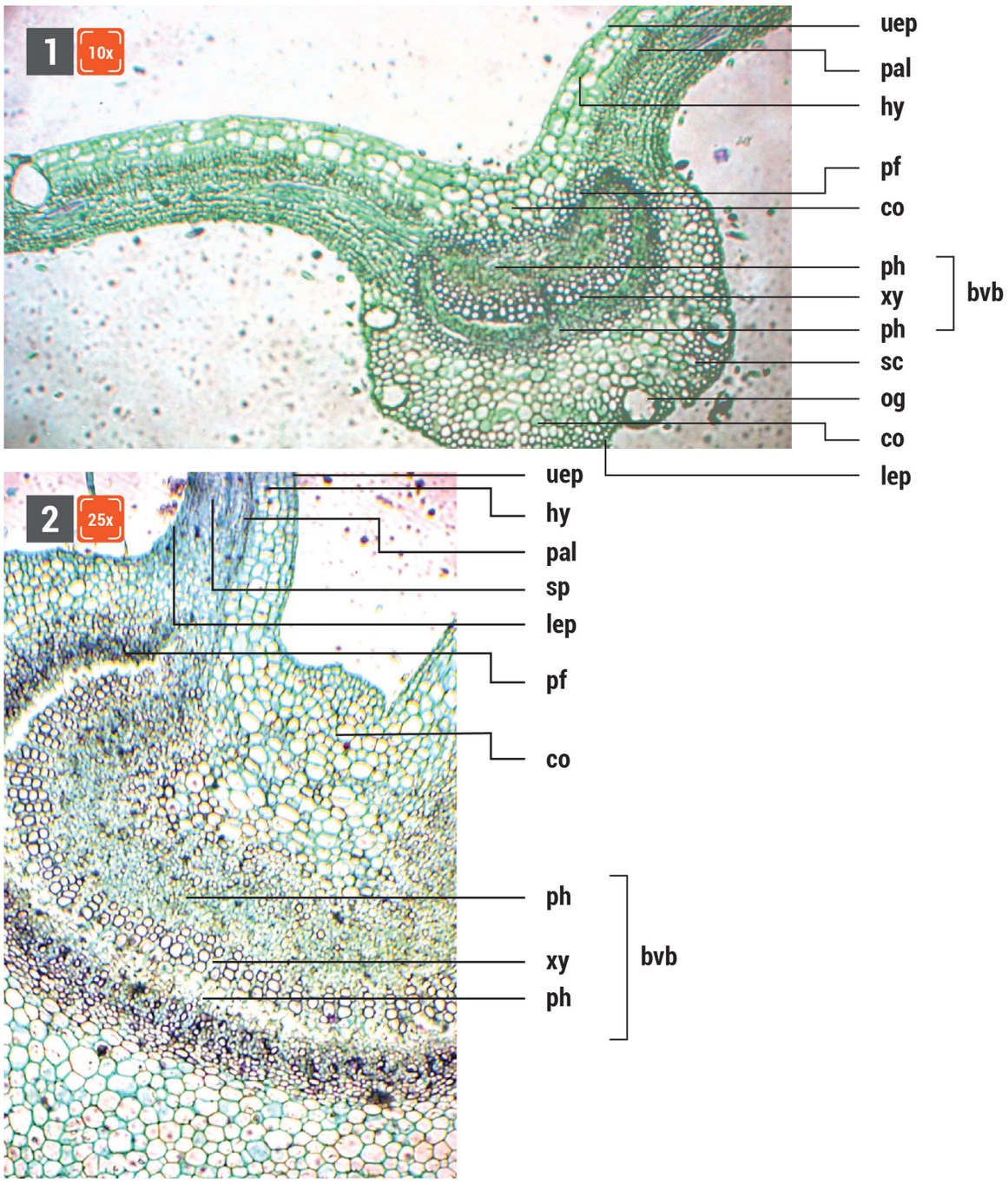
Reference HPTLC

Table 6.15. Retention Factors (R_f) and Colors of Major Bands in Sample Solution

R_f VALUE	UNDERIVATIZED			DERIVATIZED		
	NORMAL LIGHT	UV-254	UV-366	NORMAL LIGHT	UV-254	UV-366
0.74	Yellow	Black				
0.68				Purple	Black	Purple
0.61	Green	Black	Pink			
0.57			Blue			
0.53			Red			Pink
0.47			Red	Violet	Black	White/Red
0.40			Red	Pink		Pink
0.35	Brown				Black	
0.32		Black	White	Blue	Black	Blue
0.18				Blue		
0.15*	Gray	Black	Black	Brown	Black	Black
0.14						Blue

*R_f value of Gallic acid – reference standard

PSIDII GUAJAVAE FOLIUM | *Guava Leaf*
MICROSCOPIC EXAMINATION



1. Cross Section of the Leaf Blade and Midrib

2. Magnified Cross Section of Leaf Blade and Midrib

uep: upper epidermis; **pal:** palisade parenchyma; **hy:** hypodermis; **pf:** pericyclic fiber; **co:** collenchyma; **ph:** phloem; **xy:** xylem; **bvb:** bicollaterial vascular bundle; **sc:** sclerenchyma; **og:** oil gland; **lep:** lower epidermis

Annex II (DNA Barcode)

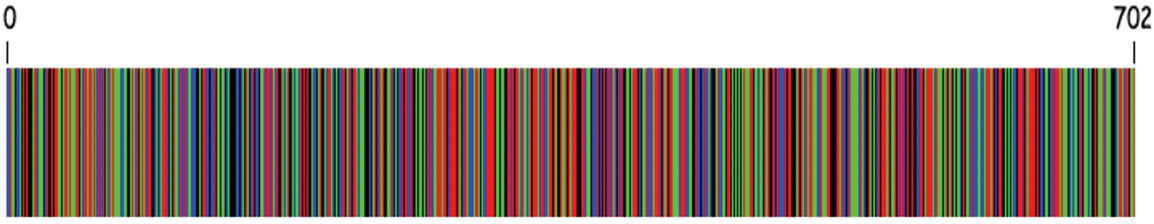
HERBAL MEDICINES

BLUMEAE BALSAMIFERAE FOLIUM | *Sambong Leaf*

***Blumea balsamifera* (L.) DC.**

> *Blumea balsamifera* voucher IBFP7 ribulose-1,5-biphosphate carboxylase/oxygenase large subunit (rbcL) gene

CTAAGCCAGTGTGGGATTCAAAGCTGGTGTTAAGATTATAAATTGACTTATTATACTCCTGA
CTATAAAACCAAGGATACTGATATCTTGGCAGCATTTCGAGTAACTCCTCAACCCGGAGTTC
CGCCTGAAGAAGCAGGGGCCGCAGTAGCTGCCGAATCTTCTACTGGTACATGGACAACTGT
GTGGACCGATGGACTTACGAGCCTTGATCGTTACAAAGGGCGATGCTATGGAATCGAGCCT
GTTCCTGGAGAAGAGTCTCAATTTATTGCTTTTGTAGCTTACCCATTAGACCTTTTTGAAGAA
GGTTCTGTTACTAACATGTTTACTTCCATTGTAGGTAATGTATTTGGGTTCAAAGCCCTGCGT
GCTCTACGTCTGGAAGATTTGCGAATCCCTATTTCGTATGTTAAAACTTTCCAAGGTCCGCCT
CACGGGATCCAAGTTGAGAGAGATAAATTGAACAAGTATGGTCGTCCCCTGTTGGGATGTA
CTATTAAACCTAAATTGGGGTTATCCGCTAAAAACTACGGTAGAGCTGTTTATGAATGTCTT
CGTGGTGGCCTTGATTTTACTAAAGATGATGAGAACGTGAACTCCCAACCATTTATGCGTTG
GAGAGACCGTTTCGTATTTTGTGCCGAAGCTCTTTATAAAGCACAAGCTGAAACAGGTGAA
ATCAAAGGGCATTACTTGAAT



PLECTRANTHI AMBOINICI FOLIUM | *Oregano Leaf*

***Plectranthus amboinicus* (Lour.) Spreng.**

> *Plectranthus amboinicus* voucher LBP2 ribulose-1,5-biphosphate carboxylase/oxygenase large subunit (rbcL) gene

GGGTGTTAAGAGTACAAATTGACTTATTATACTCCTGAATACGAAACCAAAGATACTGATAT
CTTGGCAGCATTCCGAGTAACTCCTCAACCTGGAGTTCCGCCTGAAGAAGCAGGGGCCGCG
GTAGCTGCCGAATCTTCTACTGGTACATGGACAACTGTGTGGACCGATGGACTTACCAGCCT
TGATCGTTACAAAGGGCGATGCTACCACATCGACCCCGTTCTTGGAGAAAAAGATCAATAT
ATCTGTTATGTAGCTTACCCTTTAGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACTT
CCATTGTAGGAAATGTATTTGGATTCAAAGCCCTACGTGCTCTACGTCTGGAAGATCTGCGA
ATTCCTACTGCTTATATTAAAACTTTCCAAGGCCCGCCTCATGGGATCCAAGTTGAGAGAGA
TAAATTGAACAAGTACGGTCGTCCTCTGCTGGGATGTACTATTAAACCTAAATTGGGGTTAT
CTGCTAAAAACTATGGTAGAGCGGTTTATGAATGTCTTCGCGGTGGACTTGATTTTACCAAA
GATGATGAGAACGTGAACTCCCAGCCATTTATGCGTTGGAGAGACCGCTTCTTATTTTGTGC
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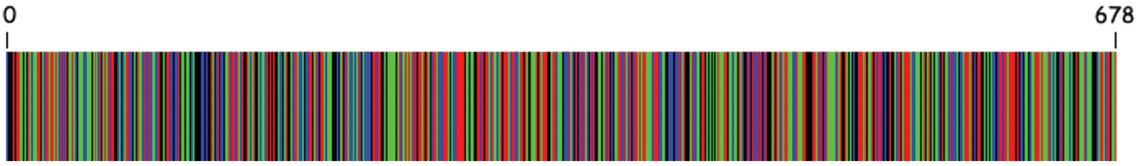

> *Plectranthus amboinicus* voucher LBP2 maturase K gene

TTTTAGAGGACAATTTTTCACATTTAAATTTTGTATTAGATATACTAATACCTCACTCTGTTCA
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ACGAGTCTTTCTCAACCAATATTGTAGTCTTATTACTCCAAAGAAAGTCAGCTCCTCTTTGTC
AAAAAGAAATCAAAGATTATTTCTTTTCTTATATAATTCTCATGTATGTGAATACGAATCTAT
TTTCGTCTTTCTACGTAACCAATCTTTTCATTTACGATCAACATCTTCTGGAGTTTTTCTTGAA
CGAATTTATTTCTATATAAAAATAGAACGTCTTGTGAACGTCTTTGTTAAGGATTTTCGGGC
GAACCTATGGTTGGTCGAGGAACCCTGCATGCATTATATTAGGTATCAAGGAAAATGCATT
CTGGCTTCAAAAGGGACATCTCTTTTCATGAATAAATGGAAATTTTACCTTGTCACTTTTTGG
CAATGGCATTTTTTGGTGTGGTTTCATCCAAGAAGGA
TTTGTATAAACCAATTTTCCAAGCATTCCCTTGAAATTTTTGGCTATCTTTCAAACGTGCAAA
CGGGCCCTTCCGTGGTACGGAGTCAGATTCTAGAAAATGCATTTCTAATCAATAATGCTATT
AAGAAGCTCGATACCCTTGTTCCAATTATTCCTCTGATTGTGAAATTGGCTAAAGATAAATTT
TGTAACGTATTGGGGCATCCCATTAGTAAGCCGATTTGGGCTGATTTATCAGATTCTAATAT
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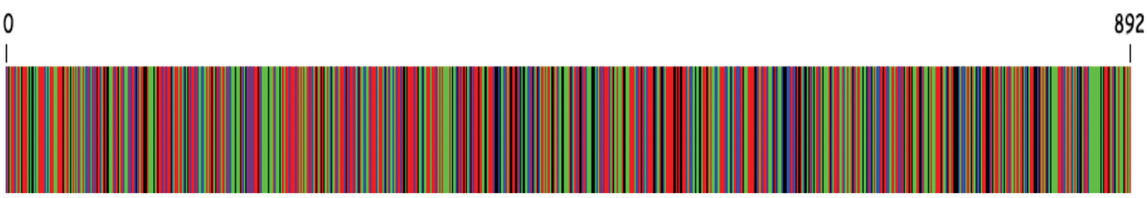
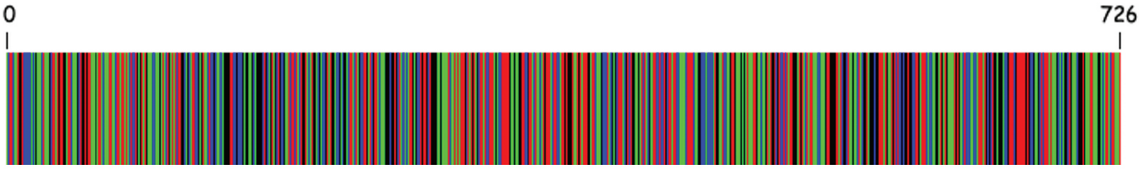


PEPEROMIAE PELLUCIDAE HERBA | *Ulasimang Bato Aerial Parts*

***Peperomia pellucida* (L.) Kunth.**

> *Peperomia pellucida* voucher (Los Baños) LBP5 ribulose-1,5-biphosphate carboxylase/oxygenase large subunit (rbcL)

CCCCCACAGAAACTAAAGCTTATGTTGGATTCAAAGCTGGTGTTAAAGATTACAAATTAACT
TATTATACTCCTGAGTATGAAACCAAAGATACTGATATCTTGGCAGCATTCCGAGTAACTCC
GCAACCAGGGGTTCCGCCCGAGGAAGCAGGGGCTGCAGTAGCTGCCGAATCTTCTACTGG
TACATGGACGACCGTATGGACCGACGGACTTACTAGCCTTGATCGTTACAAAGGGCGGTGC
TACCACATTGAGCCTGTTGCTGGGGAAGAAAATCAATATATTTGCTATGTAGCTTATCCTTT
AGACCTTTTTGAAGAAGGTTCCGTTACTAACATGTTTACTTCCATTGTGGGTAATGTATTTGG
ATTCAAAGCCCTACGAGCTTTACGTCTGGAAGATCTACGAATTCCTCCTGCTTATTCCAAAAC
TTTTCAAGGCCCACCCCATGGAATCCAAGTTGAAAGAGATAAATTGAACAAGTATGGTCGT
CCTCTATTGGGATGTACTATTAAACCAAAGTTGGGGTTATCGGCTAAGAACTACGGTAGGG
CGGTTTATGAATGTCTCCGCGGTGGCCTTGATTTCACCAAGGATGATGAAAATGTGAACTCC
CAACCATTTATGCGCTGGAGGGACCGTTTCGTTTTTTGTGCCGAAGCTCTTTATAAAGCACA
GGCTGAAACGGGTGAAATTAAAGGACATTACTTAAAT



SENNAE ALATAE FOLIUM | *Akapulko Leaf*

***Senna alata* (L.) Roxb.**

> *Senna alata* voucher (Los Baños) LBP6 ribulose-1,5-biphosphate carboxylase/oxygenase large subunit (rbcL)

CCCCACACAGAAACTAAAGCAAGTGTTGGGTTCAAAGCTGGTGTTAAAGATTATAAATTGA
CTTATTATACTCCTGACTATGAAACCAAAGATACTGATATCTTGGCAGCATTCCGAGTAACTC
CTCAACCTGGAGTTCCGCCTGAAGAAGCAGGTGCCGCGGTAGCTGCGGAATCTTCTACTGG
TACATGGACAACTGTGTGGACCGATGGGCTTACCAGTCTTGATCGTTACAAAGGACGATGC
TACCACATTGAGCCCGTTGCTGGAGAAGAAAATCAATATATTGCTTATGTAGCTTATCCCTT
AGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACTTCCATTGTGGGTAATGTATTTGG
ATTCAAGGCCCTGCGCGCTCTACGTCTGGAGGATTTGCGAATCCCTACTTCTTATATTAAAA
CTTTCCAAGGTCCGCCTCACGGCATCCAAGTTGAGAGAGATAAATTGAACAAGTACGGCCG
TCCCCTATTGGGATGTACTATTAAACCTAAATTGGGGTTATCTGCTAAGAATTATGGTAGAG
CAGTTTATGAATGTCTCCGCGGTGGACTTGATTTTACCAAAGATGATGAGAATGTGAATTCC
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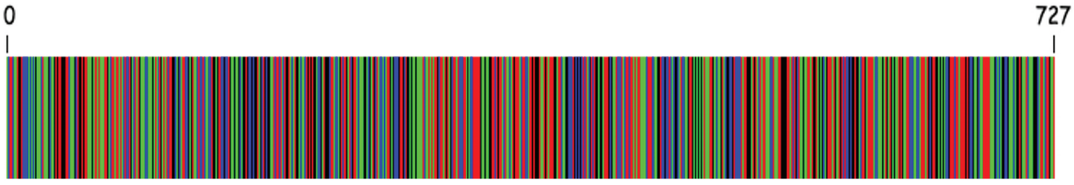
GGCCGAAACAGGTGAAATCAAAGGGCATTACTTGAAT



> *Senna alata* voucher (Nueva Ecija) NEP9 maturase K gene sequence(matK)

CAATATTTCCTTTTTTTGAGGAAAAATTTCCATATTTAAATTATGTGTCAGATGTACGAATAC
CCTACCCTATCCATCTGGAAATCTTGGTTCAAACCCTTCGATACTGGGTGAAAGATGCCTCTT
CTTTTCATTTATTAAGGCTCTTTCTTTATGAGTATTTTAATTGGAATAGTCTTATTACTCCAAA
AAAATGGATTTCTACTTTTTCAAAAAGGAATCCAAGATTATTCCTGTTCCTATATAATTTTTAT
GTATGTGAATACGAATCTATCTTTCTTTTTCTCCGTAACAAATCTTCTTATTTACGATTAACAT
CTTCTGGAGTCTTTTTTGAGCGAATCTATTTCTATGCAAAAATAGAACATTTTGTAGAAGTCT
TTGATAAAGATTTTCCGTCCACCTTATGGTTCTTCAAGGACCCTTTCATTCATTATGTTAGATA
TCAAGGAAAATCCATTTTGGCTTCAACGAATACGCCCTTTTTGATGAATAAATGGAAATACT
ATCTTATCCGTTTATGGCAATGTCATTTTTATGTTTGGTCTCAACCAGAAAAGATCCATATAA
ACCAATTATCTGAGCATTCATTTTACTTTTTGGGCTATTTTTCAAATGTGCGGCTAAATCCTTC
AGTGGTACGGAGTCAAATGCTGGAAAATTCATTTCTAATTGAAAATGTTATGAAAAGGCTT
GATACAATAATTCCAATTATTCCACTAATTAGATCATTGGCTAAAGCGAGATTTTGTAATGTA
TTAGGGCATCCCATTAGTAAGCCGGTCTGGGCCGATTCATCCGATTTGGATATTATTGACCG
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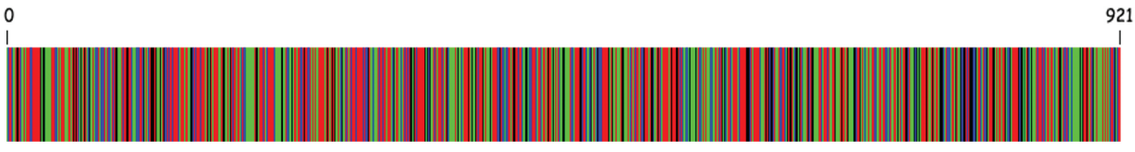

VITICIS NEGUNDONIS FOLIUM | *Lagundi Leaf*

***Vitex negundo* L.**

> *Vitex negundo* voucher (IBioFarm) IBFP5 ribulose-1,5-biphosphate carboxylase/oxygenase large subunit (rbcL) gene

TAAAGCAAGTGTTGGATTCAAAGCGGGTGTTAAAGAGTACAAATTGACTTATTATACTCCTG
AATACGAAACCAAAGATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCCGGAGTT
CCGCCTGAAGAAGCAGGGGCCGCGGTAGCTGCCGAATCTTCTACTGGTACATGGACAACTG
TGTGGACCGATGGACTTACCAGCCTTGATCGTTACAAAGGGCGATGCTACCACATCGAGCC
CGTTCTTGGAGAAAAAGATCAATATATCTGTTATGTAGCTTACCCTTTAGACCTTTTTGAAGA
AGGTTCTGTTACTAACATGTTTACTTCCATTGTAGGAAATGTATTTGGATTCAAAGCCCTGCG
TGCTCTACGTCTGGAAGATCTGCGAATCCCTACTGCTTATATTAAAACTTTCCAAGGCCCGCC
TCATGGGATCCAGGTTGAGAGAGATAAATTGAACAAGTATGGTCGTCCTCTGTTGGGATGT
ACTATTAAACCAAAATTGGGGTTATCTGCTAAAAACTATGGTAGAGCAGTTTATGAATGTCT
TCGCGGTGGACTTGATTTTACCAAAGATGATGAGAACGTGAACTCCCAGCCATTTATGCGT
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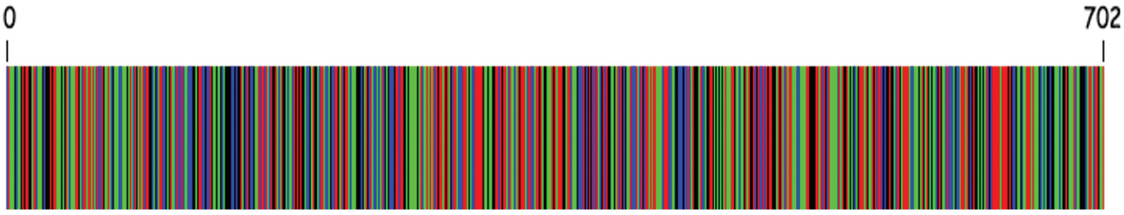
AATCAAGGGGCATTACTTGAAT



ZINGIBERIS OFFICINALIS RHIZOMA | *Luya Rhizome*

***Zingiber officinale* Roscoe.**

> *Zingiber officinale* voucher (Los Baños) LBP9 ribulose-1,5-biphosphate carboxylase/oxygenase large subunit (rbcL)

AAACTAAAGCAAGTGTTGGATTTAAAGCTGGTGTTAAAGATTACAAATTGACTTATTATACT
CCTGACTACGAAGTCAAAGATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCTGG
AGTTCCACCCGAAGAAGCAGGGGCTGCGGTAGCTGCCGAATCTTCTACTGGTACATGGACA
ACTGTGTGGACTGATGGACTTACCAGTCTTGATCGTTACAAAGGGCGATGCTACCACATCG
AGGCTGTTATTGGGGAGGATAATCAATATATTGCTTATGTAGCTTATCCTTTAGACCTTTTTG
AAGAAGGTTCTGTTACTAACATGTTTACTTCCATTGTGGGTAATGTGTTTGGTTTCAAAGCCT
TACGAGCTCTACGTTTGGAGGATCTGCGAATTCCTACTTCCTATTCCAAAACTTTCCAAGGCC
CGCCTCACGGCATTCAGGTTGAAAGAGATAAGTTGAACAAGTATGGTCGTCCCCTATTGGG
ATGTACTATTAAACCAAAATTGGGATTATCTGCAAAAAACTACGGTAGAGCAGTTTATGAGT
GTCTACGTGGTGGGCTTGATTTTACCAAAGATGATGAAAACGTGAACTCACAACCATTTATG
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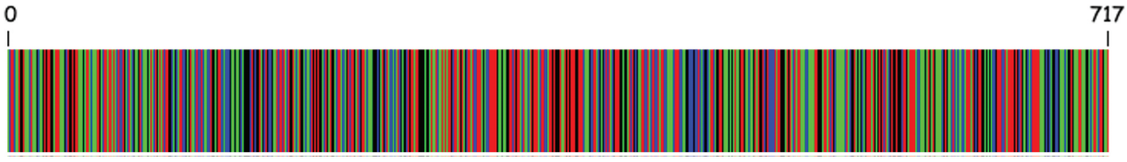
GTGAAATTAAAGGACATTACTTGAAT



MEDICINAL PLANTS

CASSIAE FISTULAE FOLIUM | *Kanya Pistula Leaf*

***Cassia fistula* L.**

> Cassia fistula voucher LBP1 ribulose-1,5-biphosphate carboxylase/oxygenase large subunit

CAACAGAAACTAAAGCAAGTGTTGGGTTCAAAGCTGGTGTTAAAGATTATAAATTGACTTA
TTATACTCCTGACTATGAAACCAAAGATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCA
ACCTGGAGTTCCGCCTGAAGAAGCAGGTGCCGCGGTAGCTGCTGAATCTTCTACTGGTACA
TGGACAACTGTGTGGACCGATGGGCTTACCAGTCTTGATCGTTACAAAGGACGATGCTACC
ACATCGAGCCCGTTGCTGGAGAAGAAAATCAATATATTGCTTATGTAGCTTATCCCTTAGAC
CTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACTTCCATTGTGGGTAATGTATTTGGGTTC
AAGGCCCTGCGCGCTCTACGTCTGGAGGATTTGCGAATCCCTATTTCTTATATTAAAACTTTC
CAAGGTCCGCCTCACGGCATCCAAGTTGAGAGAGATAAATTGAACAAGTACGGCCGTCCCC
TATTGGGATGTACTATTAAACCTAAATTGGGGTTATCCGCTAAGAATTACGGTAGAGCTGTT
TATGAATGTCTCCGCGGTGGACTTGATTTTACCAAAGATGATGAGAATGTGAATTCCCAACC
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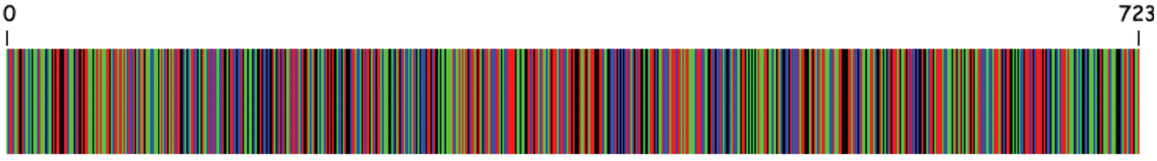
AAACAGGTGAAATCAAAGGGCATTACTTGAAT



CURCUMAE LONGAE RHIZOMA | *Luyang Dilaw Rhizomes*

***Curcuma longa* Linn.**

> *Curcuma longa* voucher IBFP8 ribulose-1,5-biphosphate carboxylase/oxygenase large subunit (rbcL) gene

AAAGCAAGTGTTGGATTTAAAGCTGGTGTTAAAGATTACAAATTGAATTATTATACTCCTGA
CTACGAAGTCAAAGATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCTGGAGTTC
CACCCGAAGAAGCAGGGGCTGCGGTAGCTGCCGAATCTTCTACTGGTACATGGACAACTGT
GTGGACTGATGGACTTACCAGTCTTGATCGTTACAAAGGGCGATGCTACCACATAGAGGCT
GTTATTGGGGAGGATAATCAATATATTGCTTATGTAGCTTATCCTTTAGACCTTTTTGAAGAA
GGTTCTGTTACTAACATGTTTACTTCCATTGTGGGTAATGTGTTTGGTTTCAAAGCCTTACGA
GCTCTACGTTTGGAGGATCTGCGAATTCCCACTTCCTATTCCAAAACTTTCCAAGGCCCGCCT
CACGGCATTCAGGTTGAAAGAGATAAGTTGAACAAGTATGGTCGTCCCCTATTGGGATGTA
CTATTAAACCAAAATTGGGATTATCTGCAAAAAACTACGGTAGAGCAGTTTATGAGTGTCTA
CGTGGTGGGCTTGATTTTACCAAAGATGATGAAAACGTGAACTCACAACCATTTATGCGTTG
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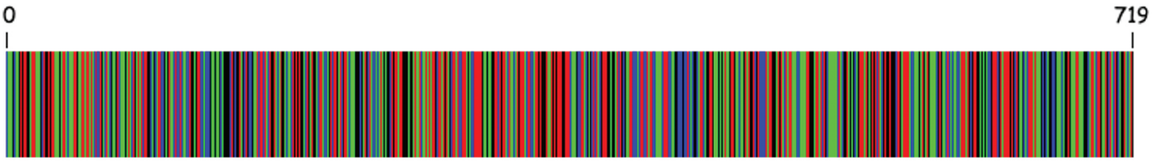
ATTAAAGGGCATTACTTGAATGCTACTGCAGGTACATG



LEUCAENAE LEUCOCEPHALAE SEMEN | *Ipil-Ipil Seeds*

Leucaena leucocephala (Lam.) de Witt

> *Leucaena leucocephala* voucher LBP12 ribulose-1,5-biphosphate carboxylase/oxygenase large subunit

CAGAAACTAAAGCAAGTGTTGGGTTCAAAGCTGGTGTTAAAGATTATAAATTGACTTATTAT
ACTCCTGACTATGAAACTAAAGATAGTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACC
TGGAGTTCCGCCTGAAGAAGCGGGTGCCGCGGTAGCTGCTGAATCTTCTACTGGTACATGG
ACAACTGTGTGGACCGATGGGCTTACCAGTCTTGATCGTTACAAAGGACGATGCTACCACA
TCGAGCCAGTTGCTGGAGAAGAAAGTCAATTTATTGCTTATGTAGCTTATCCCTTAGACCTT
TTTGAAGAAGGTTCTGTTACTAACATGTTTACTTCCATTGTGGGTAATGTATTTGGGTTCAAG
GCCCTGCGTGCTCTACGTCTGGAGGATTTGCGAATCCCTCCTTCTTATTCTAAAACTTTCCAA
GGTCCGCCTCACGGCATCCAAGTTGAGAGGGATAAATTGAACAAGTATGGCCGTCCCCTAT
TGGGATGTACTATTAAACCAAAATTGGGGTTATCCGCTAAGAATTACGGTAGAGCGGTTTA
TGAATGTCTCCGTGGTGGACTTGATTTTACCAAAGATGATGAGAATGTGAATTCCCAACCAT
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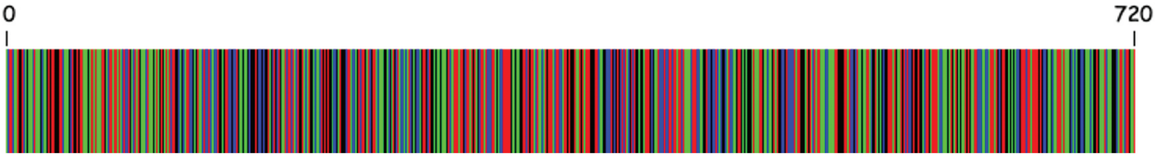
ACAGGTGAAATCAAAGGGCATTACTTGAAT



MORINGAE OLEIFERAE FOLIUM | *Malunggay Leaf*

***Moringa oleifera* Lam.**

> *Moringa oleifera* voucher (Los Banos) LBP7 ribulose-1,5-biphosphate carboxylase/oxygenase large subunit

CACCMACAACAGAAACTAAAGCAAGTGTTGGATTCAAAGCTGGTGTTAAAGATTATAAATT
GACTTATTATACTCCTGACTATGAAACCAAAGATACTGATATCTTGGCAGCATTCCGAGTAA
CTCCTCAACCCGGAGTTCCACCTGAGGAAGCCGGGGCCGCGGTAGCTGCTGAATCTTCTAC
TGGTACATGGACAACTGTGTGGACCGATGGGCTTACCAGCCTTGATCGTTACAAAGGACGA
TGCTACCACATCGAGCCCATTGCGGGAGAAGAAAATCAATTTATTGCTTATGTAGCTTACCC
CTTAGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACTTCTATTGTGGGTAATGTATT
TGGGTTCAAAGCCCTGCGCGCTCTACGTCTAGAGGATCTGCGAATCCCTCCTGCTTATTCTA
AAACTTTCCAGGGACCACCTCATGGTATCCAAGTTGAAAGAGATAAATTGAACAAGTATGG
CCGTCCCCTATTAGGATGTACTATTAAACCTAAATTGGGTTTATCCGCTAAGAACTACGGTA
GAGCGGTTTATGAATGTCTACGCGGTGGACTTGATTTTACCAAAGATGATGAGAATGTGAA
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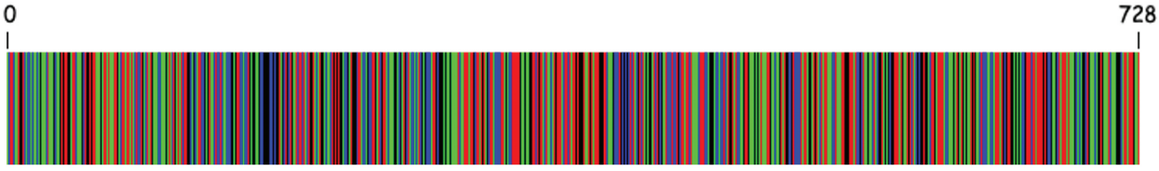
ACAGGCTGAAACAGGTGAAATCAAAGGGCATTATTTGAAT



> *M. oleifera* voucher (Los Banos) LBP7 maturase K gene sequence(matK)

TATTCCCTTTTTAGAGGACAAATTTTCACATTTAAATTATGTATTAGATGTACTAATCCCTTAC
CCCATCCATCTGGAAATCTTGGTTCAACCCCTTCGTTACTGGGTAAAAGATGCCTCTTCTTTG
CATTTATTACGGTTCTGTCTCTACAAGTATTGTAATTGGAAGAGTCTTATTACTCCAAAGAAA
TCTTTTTTTAATCCAAGGTTTTTCTTGTTCTTATATAATTCTCATGTATGTGAATACGAATCCA
TCTTATTTTTTCTCCGCAACCAATCTTCTCATTTACGATCAACATCTTCTGGAGTCCTTCTTGA
GCGCATTTTTTTCTATGGAAAAATAGAACATCTTATAAAAACACATCTTATAAAAACCTTTGT
TAATGGTTTTCAGGACATCTTATGCTTGTTCAAGGATCCTTTCATGCATTATGTTAAATATCA
AGGAAAATCCATTTTGGCCTCAAAAGATACGCCTCTTCTGATGAATAAATGGAAATATTACT
TTGTCAATTTATGGCAATGTCATTTTTACGTGTGGTTTCAATCACGAAGGGTCCGTATAAACC
AATTATCCAAAGATTATCTAGACTTTCTGGGCTATTTTTCAAGTTTGCGATTTAATCCGTTAG
TGGTACGGAGCCAAATGCTAGAAAACTCATTTATAATAGATAATGCTATAAAGAAGTTCGA
TACAAAAATTCCAATTATTCCTATGATTGGATCATTGGCTAAAGCGAGATTTTGTAATACATT
AGGGCATCCCATTAGTAAGCCGACCTGGGCCGATTCATCAGATTCTGATATTATCGACCGAT
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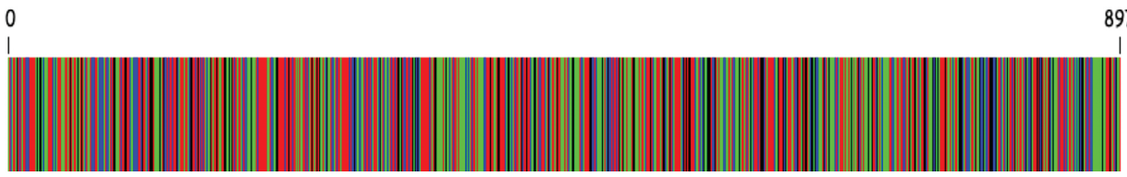
TATCGAAT

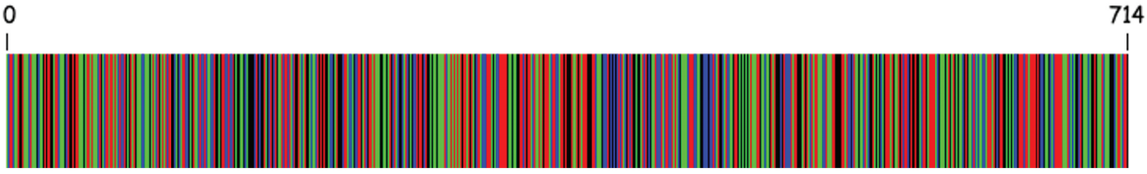


PSIDII GUAJAVAE FOLIUM | *Guava Leaf*

Psidium guajava L.

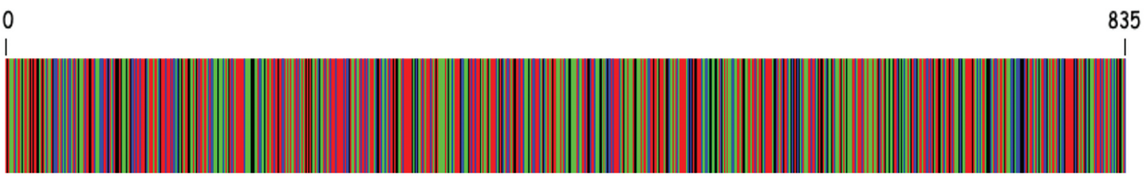
> *Psidium guajava* voucher (Los Banos) LBP11 ribulose-1,5-biphosphate carboxylase/oxygenase large subunit

AAACTAAAGCAAGTGTTGGATTCAAAGCTGGTGTTAAAGATTATAAACTGACTTATTATACT
CCTGAGTATGAAACCAAAGATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCTGG
AGTTCCTCCTGAGGAAGCAGGGGCTGCGGTAGCTGCTGAATCTTCTACTGGTACATGGACA
ACTGTGTGGACCGATGGGCTTACCAGCCTTGATCGTTATAAAGGAAGATGCTACGGCATCG
AGCCTGTTGCTGGAGAAGAAAATCAATATATATGTTATGTAGCTTACCCTTTAGACCTTTTTG
AAGAAGGTTCTGTTACTAATATGTTTACTTCCATTGTGGGTAATGTATTTGGGTTCAAAGCC
CTGCGCGCTCTACGTCTGGAGGATCTGCGAATCCCTACTTCCTATACGAAAACTTTCCAAGG
CCCGCCTCATGGCATCCAAGTTGAGAGAGATAAATTGAACAAGTATGGGCGTCCCCTATTG
GGATGTACTATTAAACCTAAATTGGGGTTATCCGCTAAGAACTACGGTAGAGCAGTTTATG
AATGTCTTCGTGGTGGACTTGATTTTACGAAAGATGATGAGAACGTGAACTCACAACCATTT
ATGCGTTGGAGAGACCGTTTCTTATTTTGTGCCGAAGCACTTTTTAAATCACAGGCTGAAAC
AGGTGAAATCAAAGGGCATTACTTG



> *Psidium guajava* voucher (Los Banos) LBP11 maturase K (matK)

GATTATGTGTTGGATGTACTAATGCCCTATCACATCCATCTAGAAATCTTGGTTCAAACCCTT
CGCTACTGGGTGAAAGATGCCTCTTCTTTGCATTTATTACGTTTCTTTCTCCACGAGTATTGG
AATAGTCTTATTACTCCAAAGAAACATAGTCCTATTTTTTCAAAAGGGAATCCAAGATTATTC
TTGTTCCTATATAATTCTCATATGTGTGAATACGAATCTATCTTTCTTTTTCTCCGTAATCAAT
CTTCTCATTTACGGTCAACATCTTGTGGAATCTTTTTTGAGCGAATCTATTTCTATGTAAAAAT
AGAACATTTTGCCAAAGTCTTCTTTGATAATGATTTTCAGTGCATCCTATGGTTCTTCAAAGA
TCCTTTCATGCATTATGTTAAATATCAAGGAAAATCAATTCTGGCTTCAAAGGATACGCCTCT
TTTCATGAATAAATGGAAATATTACCTTGTTAGTTTATGGCAATATCATTTTTACGCGTGGTT
TCAACCAGGAAGGATCGATATAAACCAATTATGGAATTATTCTATTGACTTTTTAGGCTATC
GTTCAAGCGTGCGACTAAATTCTTCAGTGGTACGAAGTCAAATGATAGAAAATTCATTTCTA
ATAAATAATGCTATGAAGAAGTTCGAGACAATAGTTCCGATTATTCCTCTGATTGGATCATT
GTCTAAAGCGAATTTTTGTAACACATTAGGGCATCCTATTAGTAAACCGACCCGGGCTGATT
CATCAGATTCCGATATTATCGACCGTTTTTTGCGTATATGCAGAAATCTTTCTCATTATCACA
GTGGATC



OTHERS

MENTHA SPICATA L. | *Yerba Buena*

> M*entha spicata* voucher (Los Baños) LBP4 maturase K gene (matK)

TTTGTGTTAGATATACTAATACCTCGCTCTGTCCATGTGGAAATCTTGATTCAAACTCTTCGC
CATTGGGTAAAAGATGTTTCTTCTTTACATTTATTACGGGTCTTTCTCAACGAATATTGGAAT
TGGAATAGTCTTCTTACTCCAAAGAAAGTCAGCTTCTCTTTGTCAAAAAGAAATCAAAGGTT
ATTTTTTTTCTTATATAATTCTCATGTATGTGAATACGAATCTATTTTCGTCTTTCTACGTAACC
AATCTTTTCATTTACGATCAACATCTTCTGGAGTTCTTCTTGAACGAATCTATTTCTATATAAA
AATAGAACGTCTTATGAACGTCTTTGTTAAGGATTTTCGGGCGAACCTATGGTTGGTCGAG
GAACCCTGCATGCATTATATTAGGTATCAAAGAAAATCCATTCTGGCTTCCAAAGGGACATC
CCTTTTCATGAATAAATGGAAATTAAACCTTGTCACTTTTTGGCAATGGCATTTTTCTGTGTG
GTTTCATCCAAGAAGGATTTGGATAAACCAATTTCCCAAGCATTCCCTTGAAATTTTGGGTT
ATCTTTCAAACGTGCAAATGAACCCTTCCGTGGTACGGAGTCAAATTCTAGAAAATTCATTT
CTAATCAATAATGCTATTAAGAAGCTCGATACTCTTGTTCCAATTATTCCTCTGATTGCGGAA
TTGGCTAAAGCTAAATTTTGTAATGTATTGGGGCATCCCATTAGTAAGCCGATTCGGGCTGA
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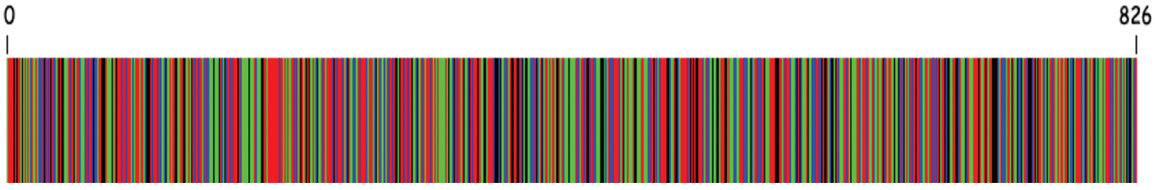
AGCGGATCT



LAGERSTROEMIA SPECIOSA (L) PERS | *Banaba*

> *Lagerstroemia speciosa* voucher (Los Banos) LBP3 ribulose-1,5-biphosphate carboxylase/oxygenase large subunit (rbcL) gene

AAAGCTTTTGTTGGATTCAAAGCCGGTGTTAAAGATTATAAACTGACTTATTATACTCCTAAC
TATCAAACCAAAGATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCTGGTGTTCC
GCCTGAGGAAGCAGGGGCTGCAGTAGCTGCTGAATCTTCTACTGGTACCTGGACAACTGTG
TGGACCGATGGGCTTACCAGCCTTGATCGTTATAAAGGAAGATGCTACAACATCGAGCCTG
TTGCTGGAGAAGAAAATCAATATATATGTTATGTCGCTTACCCTTTAGACCTTTTTGAAGAA
GGTTCTGTTACTAATATGTTTACTTCCATTGTGGGTAATGTATTTGGGTTCAAAGCCCTGCGC
GCTCTACGTCTGGAGGATCTGAGAATCCCTACTGCCTATATTAAAACTTTCCAAGGCCCGCC
TCATGGTATCCAAGTTGAGAGAGATAAATTGAACAAGTATGGCCGTCCCCTATTGGGATGT
ACTATTAAACCTAAATTGGGATTATCTGCTAAGAACTACGGTAGAGCGGTTTATGAATGTCT
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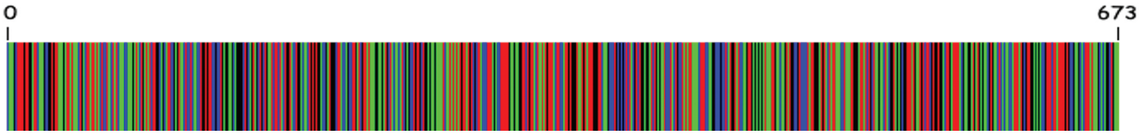

> *Lagerstroemia speciosa* voucher (Nueva Ecija) NEP10 maturase K (matK)

CGCTACTGGGTGAAGGATGCCTCTTCTTTGCATTTATTACGTTTCTTTTTCTAGAGTATTGTAATT
GGAATAGTCTTATTACTCCCCAAAAACATATTTCCATTTTTTTAAAAGGTAATCCAAGATTATTCT
TGTTCCTATATAATTCTTATGTATGTGAATACGAATCCATCTTCCTTTTTCTCCGTAATCAATCTTC
TCATTTCCGGCCAACATCTTCTGGAGTCTTTTTTGAGCGAATATATTTCTATGTAAAAATAGAACT
TCCTGTTGAAGTTTTTTTTGATAATGATTTTCGGGACATTCGAGCCTTCTTCAAGTATTCTTTCAT
GCATTATGTTAGATATCAAGGAAAATCAATTCTGGCTTCGAAAGATACACCCTTTCTGATGAATA
AATGGAAATATTACCTTGTCAATTTATGGCAATATCGTTTTTACGCGTGGTCTCAACCAGGAAGG
ATCAATCTAAACCAATTAGGAAAGTATTCTCTTGACTTTTTGGGCTATTTTTCAAACGTGCAACTA
ACTTTTTCAGTGGTACGAAGTAAAATGCTAGAAAAATCATTTCTGATAAATACTGCTATGAAGAA
GCTCGAAACAACAGTTCCAATTATTCCTTTGATTGGATCTTTGTCTAAAGCGAAATTTTGTAACGT
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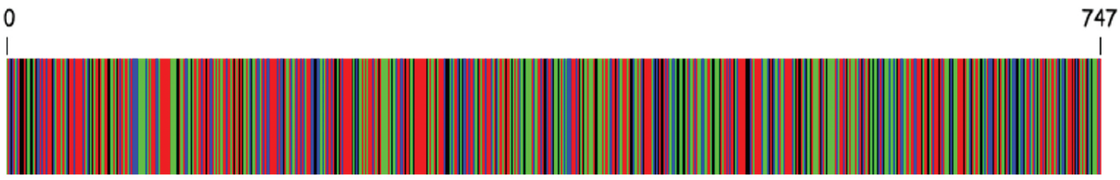

OCIMUM GRATISSIMUM L. | *African Basil*

> *Ocimum gratissimum* voucher (Los Banos) LBP2 maturase K gene (matK)

TTTTAGAGGACAATTTTTCACATTTAAATTTTGTATTAGATATACTAATACCTCCTCTGTTCAT
GTGGAAATCTTGATTCAAACTCTTCGCTATTGGGTAAAAGATGTTTCTTCTTTGCATTTATTA
CGAGTCTTTCTCAACCAATATTGTAGTCTTATTACTCCAAAGAAAGTCAGCTCCTCTTTGTCA
AAAAGAAATCAAAGATTATTTCTTTTCTTATATAATTCTCATGTATGTGAATACGAATCTATT
TTCGTCTTTCTACGTAACCAATCTTTTCATTTACGATCAACATCTTCTGGAGTTTTTCTTGAAC
GAATTTATTTCTATATAAAAATAGAACGTCTTGTGAACGTCTTTGTTAAGGATTTTCGGGCG
AACCTATGGTTGGTCGAGGAACCCTGCATGCATTATATTAGGTATCAAGGAAAATGCATTCT
GGCTTCAAAAGGGACATCTCTTTTCATGAATAAATGGAAATTTTACCTTGTCACTTTTTGGCA
ATGGCATTTTTTGGTGTGGTTTCATCCAAGAAGGATTTGTATAAACCAATTTTCCAAGCATTC
CCTTGAAATTTTTGGCTATCTTTCAAACGTGCAAACGGGCCCTTCCGTGGTACGGAGTCAGA
TTCTAGAAAATGCATTTCTAATCAATAATGCTATTAAGAAGCTCGATACCCTTGTTCCAATTA
TTCCTCTGATTGTGAAATTGGCTAAAGATAAATTTTGTAACGTATTGGGGCATCCCATTAGT
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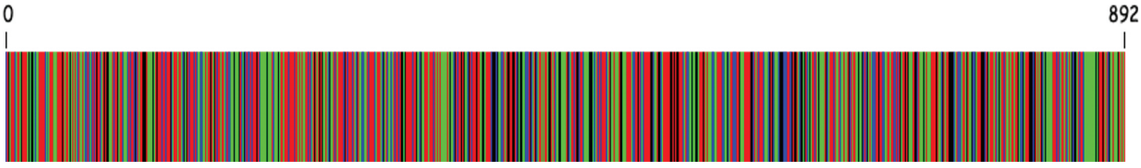

OCIMUM TENUIFLORUM L. | *Holy Basil*

> *Ocimum tenuiflorum* voucher (Nueva Ecija) NEP8 ribulose-1,5-biphosphate carboxylase/oxygenase large subunit (rbcL) gene

CACAACAGAAACTAAAGCAAGTGTTGGATTCAAAGCGGGTGTTAAAGAGTACAAATTGACT
TATTATACTCCTGAATACGAAACCAAAGATACTGATATCTTGGCAGCATTCCGAGTAACTCC
TCAACCTGGAGTTCCGCCTGAAGAAGCAGGGGCCGCGGTAGCTGCCGAATCTTCTACTGGT
ACATGGACAACTGTGTGGACCGATGGACTTACCAGCCTTGATCGTTACAAAGGGCGATGCT
ACCACATCGACCCCGTTCTTGGAGAAAAAGATCAATATATCTGTTATGTAGCTTACCCTTTA
GACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACTTCCATTGTAGGAAATGTATTTGGA
TTCAAAGCCCTACGTGCTCTACGTCTGGAAGATCTGCGAATTCCTACTGCTTATATTAAAACT
TTCCAAGGCCCGCCTCATGGGATCCAAGTTGAGAGAGATAAATTGAACAAGTACGGTCGTC
CTCTGCTGGGATGTACTATTAAACCTAAATTGGGGTTATCTGCTAAAAACTATGGTAGAGCG
GTTTATGAATGTCTTCGCGGTGGACTTGATTTTACCAAAGATGATGAGAACGTGAACTCCCA
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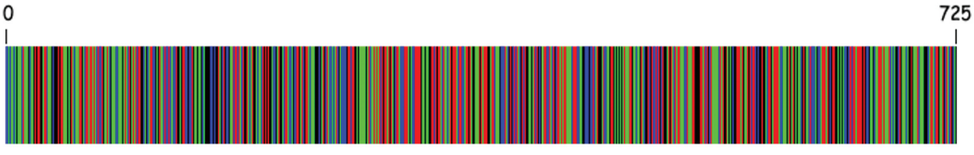

THLADIANTHA HENRYI HEMSL. | *Golden Creeper*

> *Thladiantha henryi* voucher (Los Banos) LBP13 ribulose-1,5-biphosphate carboxylase/oxygenase large subunit (rbcL)

MCAACAGAAACTAAAGCAAGTGTTGGATTCAAAGCTGGTGTTAAAGATTATAAATTGACTT
ATTATACTCCTGAATATGAAACCAAAGATACTGATATCTTGGCAGCATTCCGAGTAACTCCT
CAACCGGGAGTTCCACCCGAGGAAGCAGGGGCCGCTGTAGCTGCTGAATCTTCTACTGGTA
CATGGACAACTGTGTGGACCGATGGGCTTACCAGTCTTGATCGTTACAAAGGACGATGCTA
TGGCATCGAGCCTGTTCCTGGAGAAGAAAGTCAATTTATTGCTTATGTAGCTTATCCCCTAG
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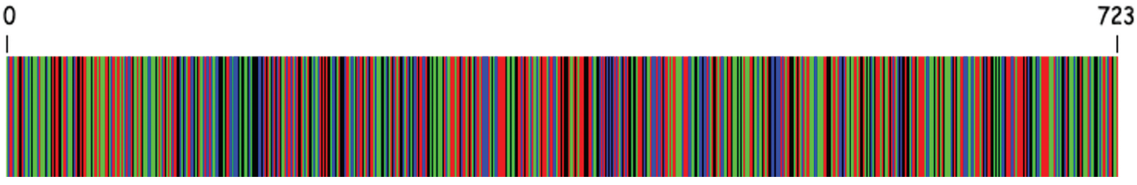

CLERODENDRANTHUS SPICATUS (THUNB.) C.Y.WU or ORTHOSIPHON ARISTATUS (BLUME) MIQ. | *Balbas Pusa*

> *Clerodendranthus spicatus* voucher (IBioFarm) IBFP4 ribulose-1,5-biphosphate carboxylase/oxygenase large subunit (rbcL) gene

TAAAGCAAGTGTTGGATTCAAAGCGGGTGTTAAAGAGTACAAATTGACTTATTATACTCCTG
AATACGAAACCAAAGATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCTGGAGTT
CCGCCTGAAGAAGCAGGGGCCGCGGTAGCTGCCGAATCTTCTACTGGTACATGGACAACTG
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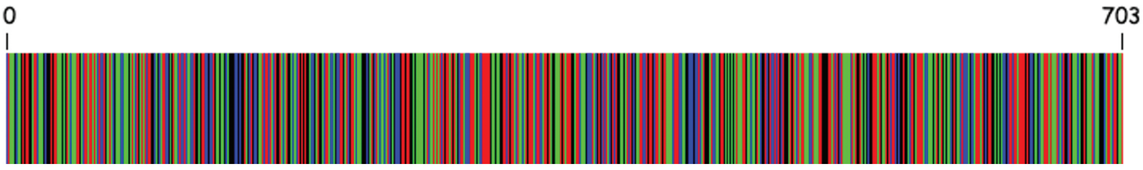

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